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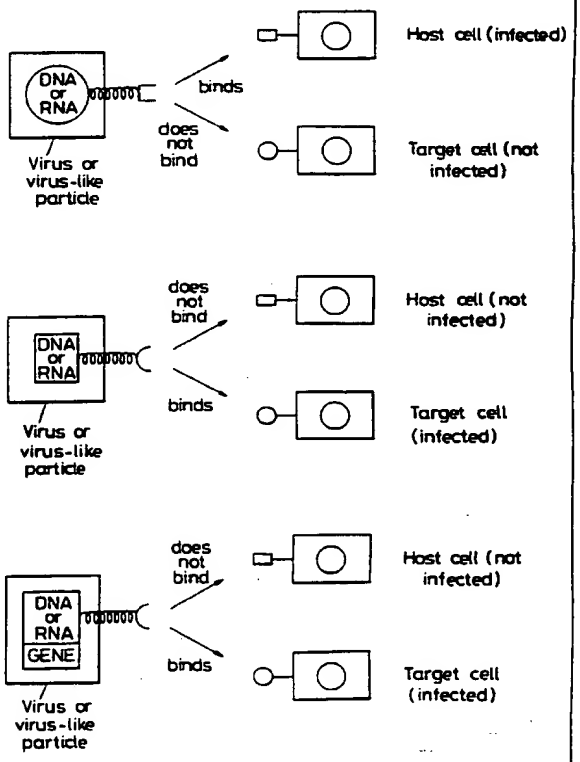
(57) Abstract

A virus, or virus-like particle, derived from a virus or virus-like particle having a receptor for a host cell comprising a modified binding specificity conferred by a binding moiety allowing the virus or virus-like particle to bind to a target cell characterised in that the said host cell receptor is modified or absent so that the virus or virus-like particle is substantially incapable of binding the said host cell are disclosed. An adenovirus or influenza virus or vaccinia virus, or a replication defective derivative of any of these, characterized in that the virus has a modified binding specificity conferred by a binding moiety allowing the virus to bind to a target cell are disclosed. Suitable binding moieties include monoclonal antibodies, ScFvs, dAbs and minimal recognition units. The use of at least some of these as delivery vehicles for genes to target cells in the fields of gene therepy and cancer treatment are disclosed.



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VIRUS WITH MODIFIED BINDING MOIETY SPECIFIC FOR THE TARGET CELLS.

The present invention relates to delivery vehicles for genes to target cells, especially in the fields of gene therapy and cancer treatment.

The delivery of genes to target cells, especially those within the mammalian body, has many uses, for example in the fields of gene therapy, cancer treatment and in areas of genetic manipulation still to be discovered. The gene to be delivered may encode a molecule, such as a protein or RNA, which is cytotoxic to the target cell, or it may encode a functional copy of a gene that is defective in the target cell. In this latter case the product of the aforementioned functional copy of the gene will replace that of the defective copy, and the target cell will be able to perform its proper function.

The use of viruses, or virus-like particles, to deliver genes for gene therapy and cancer treatment has been disclosed.

However, in most cases the targeting of the virus or virus-like particles containing the desired gene to the cell has relied on the natural host-virus specificity or on local application of the virus to the cells to be targeted, for example direct application of viruses to lung cells by inhalation.

The human adenovirus 5 (Ad5) genome consists of a double-stranded linear DNA molecule of 36 kilo-basepair. The virus replication cycle has two phases: an early phase, during which four transcriptional units E1, E2, E3, and E4 are expressed, and a late phase occurring after the onset of viral DNA synthesis when late transcripts are expressed from the major late promoter (MLP). These late messages encode most of the viral structural proteins. E1, E2, and E4 gene products of human adenoviruses

(Ads) are involved in transcriptional activation, cell transformation, and viral DNA replication as well as other viral functions, and are essential for viral growth. In contrast, E3 gene products are not required for viral replication in cultured cells or for acute lung infection of cotton rats, but appear to be involved in evading immune surveillance *in vivo*.

By "virus-like particle" we mean a nucleoprotein particle containing a core of nucleic acid surrounded by protein which (i) is not infective and (ii) can only be propagated in a suitable cell system following transformation by its nucleic acid. Thus a virus-like particle of mammalian origin may be propagated in *Saccharomyces cerevisiae* or in insect cells *via* a baculovirus expression system.

The modification of coat proteins of filamentous bacteriophages (bacterial viruses), such as M13 and fd, so as to generate novel binding properties, has been disclosed in Cwirla *et al* (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6378-6382 and Scott & Smith (1990) *Science* **249**, 386-390.

It has previously been suggested that retroelement particles, including retroviral vectors, may be modified to target specific cells, for example see Kingsman *et al* (1991) *Tibtech* **9**, 303-309.

Russell *et al* (1993) *Nucl. Acids Res.* **21**, 1081-1085, published after the priority date for this application but before the filing date discloses retroviral vectors displaying functional antibody fragments and suggests that, in principle, the display of antibody fragments on the surface of recombinant retroviral particles could be used to target virus to cells for gene delivery. However, it is not known whether a retrovirus can be assembled in which all the subunits of the viral envelope protein are fused to antibody, and if so whether the virus would infect cells.

NIP-derivatised human cells were tested as a method for targeted gene delivery, but became permissive for both modified (displaying an anti-NIP antibody) and unmodified ecotropic viral particles. NIP is 4-hydroxy-3-iodo-5-nitrophenylacetic acid.

Michael *et al* (1993) *J. Biol. Chem.* 268, 6866-6869, published after the priority date of this application but before the filing date, describes molecular conjugates between adenovirus and a vector system comprising two linked domains, a DNA binding domain and a ligand domain. In this configuration, however, it is stated that the viral moiety functions in the capacity of both an alternate ligand domain of the conjugate and, since an additional ligand has been introduced into the conjugate design, the potential for cell-specific targeting is undermined.

Curiel *et al* (1992) *Human Gene Therapy* 3, 147-154 describes adenoviruses wherein a foreign epitope was introduced into the hexon protein and polylysine-antibody complexed DNA was attached to adenovirus by virtue of the antibody binding the foreign epitope on the hexon. Foreign DNA is transferred bound to the exterior of the virion.

The above-mentioned viruses and virus-like particles may be able to target cells using the binding moiety displayed on their surface but they can also still target their natural host cells.

We have now devised new viruses and virus-like particles at least some of which can bind the target cell with high specificity and may deliver genetic material to the target cell; at least some of the viruses and virus-like particles may bind and deliver genetic material to the target cell without substantially binding to the natural host cell of the virus.

One aspect of the present invention provides a virus, or virus-like particle, derived from a virus or virus-like particle having a receptor for a host cell comprising a modified binding specificity conferred by a binding moiety allowing the virus or virus-like particle to bind to a target cell characterised in that the said host cell receptor is modified or absent so that the virus or virus-like particle is substantially incapable of binding the said host cell.

By "substantially incapable of binding its host cell" we mean that the modified virus has no more than 1% of the binding affinity of the unmodified virus for the host cell.

In general, the binding specificity of a natural virus or virus-like particle is conferred by the specific interaction between a receptor-like molecule expressed on the surface of the virus or virus-like particle and a cognate receptor-like molecule expressed on the surface of its host cell. The invention provides a beneficial modification of the binding specificity, so that the virus or virus-like particle can bind to a different specific target cell.

The introduction of the modified binding moiety may be such as to achieve the said removal of the native binding specificity.

A second aspect of the invention comprises an adenovirus or influenza virus or vaccinia virus, or a replication-defective derivative of any of these, characterised in that the virus has a modified binding specificity conferred by a binding moiety allowing the virus to bind to a target cell.

By "binding moiety" we mean a molecule that is exposed on the surface of the virus or virus-like particle which is able to bind to a molecule on

the target cell. The "binding moiety" may be a molecule on the virus or virus-like particle modified in such a way that its binding specificity is changed, or it may be a molecule added to, and exposed on the surface of, the virus or virus-like particle to provide a new binding specificity.

It is preferred if the binding moiety is external to the receptor for its host cell of the naive, unmodified virus.

It is further preferred if the binding moiety is joined or fused to the virus or virus-like particles directly or indirectly by a spacer group.

By "host cell" we mean the cell that an unmodified, naive virus can bind to using its receptor-like molecule and the cognate receptor-like molecule on the cell. By "target cell" we mean the cell that the modified virus can bind to using its binding moiety. In some circumstances in the context of the second aspect of the invention, such as when the binding moiety recognises an entity on the host cell which is not the cognate receptor-like molecule, then the host cell may be the target cell.

The virus or virus-like particle may be a bacteriophage and the target cell a bacterium in which case the invention may find uses in the treatment of bacterial infections.

In a preferred embodiment of the invention the target cell is eukaryotic. The eukaryotic cell may be a yeast cell and the virus or virus-like particle may be useful in the medical field in treating yeast infections such as athlete's foot or *Candida* infection but it is preferred that the eukaryotic cell is mammalian, and it is expected that the invention will find uses in the areas of gene therapy and cancer treatment.

In preferred embodiments of the first aspect of the invention the virus or virus-like particle is adenovirus or influenza virus or a pox-virus such as vaccinia.

It is also preferred that the virus or virus-like particle is "replication-defective". By "replication defective" we mean a virus whose genetic material has been manipulated so that it cannot divide or proliferate in the cell it infects.

The binding moiety of the virus or virus-like particle of the invention provides the target cell binding specificity. Any cell-binding protein or peptide or carbohydrate or lipid may be useful for targeting the virus or virus-like particle to the cell. For example, short linear stretches of amino acids, such as those constituting a peptide hormone, are useful, as are domains of polypeptides that can fold independently into a structure that can bind to the target cell.

In one preferred embodiment the binding moiety has the property of any one of a monoclonal antibody, ScFv (single chain Fv fragment), a dAb (single domain antibody) or a minimal recognition unit of an antibody.

The binding site on the target cell may be a target cell-specific antigen. Such antigens are listed in Table 1. Other binding moieties, targets on cells, and diseases which could usefully be treated using reagents delivered by the modified viruses or virus-like particles are given in Table 2.

Table 1

1. Tumour Associated Antigens

Antigen	Antibody	Existing Uses
Carcino-embryonic Antigen	{C46 (Amersham) {85A12 (Unipath)	Imaging & Therapy of colon/rectum tumours.
Placental Alkaline Phosphatase	H17E2 (ICRF, Travers & Bodmer)	Imaging & Therapy of testicular and ovarian cancers.
Pan Carcinoma	NR-LU-10 (NeoRx Corporation)	Imaging & Therapy of various carcinomas incl. small cell lung cancer.
Polymorphic Epithelial Mucin (Human milk fat globule)	HMFG1 (Taylor-Papadimitriou, ICRF)	Imaging & Therapy of ovarian cancer, pleural effusions.

β-human Chorionic Gonadotropin	W14	Targeting of enzyme (CPG2) to human xenograft choriocarcinoma in nude mice. (Searle *et al* (1981) *Br. J. Cancer* **44**, 137-144)
A Carbohydrate on Human Carcinomas	L6 (IgG2a)[1]	Targeting of alkaline phosphatase. (Senter *et al* (1988) *P.N.A.S.* **85**, 4842-4846
CD20 Antigen on B Lymphoma (normal and neoplastic)	1F5 (IgG2a)[2]	Targeting of alkaline phosphatase. (Senter *et al* (1988) *P.N.A.S.* **85**, 4842-4846

[1]Hellström *et al* (1986) *Cancer Res.* **46**, 3917-3923

[2]Clarke *et al* (1985) *P.N.A.S.* **82**, 1766-1770

Other antigens include alphafoetoprotein, Ca-125 and prostate specific antigen.

2. Immune Cell Antigens

Pan T Lymphocyte Surface Antigen (CD3)	OKT-3 (Ortho)	As anti-rejection therapy for kidney transplants.

B-lymphocyte Surface Antigen (CD22)	RFB4 (Janossy, Royal Free Hospital)	Immunotoxin therapy of B cell lymphoma.
Pan T lymphocyte Surface Antigen (CD5)	H65 (Bodmer, Knowles ICRF, Licensed to Xoma Corp., USA)	Immunotoxin treatment of Acute Graft versus Host disease, Rheumatoid Arthritis.

3. Infectious Agent-Related Antigens

Mumps virus-related	Anti-mumps polyclonal antibody	Antibody conjugated to Diphtheria toxin for treatment of mumps.
Hepatitis B Surface Antigen	Anti HBs Ag	Immunotoxin against Hepatoma.

Table 2: Binding moieties for tumour-specific targets and tumour associated antigens

Target	Binding moiety	Disease
Truncated EGFR	anti-EGFR mAb	Gliomas
Idiotypes	anti-id mAbs	B-cell lymphomas
EGFR (*c-erb*B1)	EGF, TGFα anti-EGFR mAb	Breast cancer
*c-erb*B2	mAbs	Breast cancer
IL-2 receptor	IL-2 anti-Tac mAb	Lymphomas and leukaemias
IL-4 receptor	IL-4	Lymphomas and leukaemias
IL-6 receptor	IL-6	Lymphomas and leukaemias
MSH (melanocyte-stimulating hormone) receptor	α-MSH	Melanomas
Transferrin receptor (TR)	Transferrin anti-TR mAb	Gliomas
gp95/gp97	mAbs	Melanomas
p-glycoprotein cells	mAbs	drug-resistant
cluster-1 antigen (N-CAM)	mAbs	Small cell lung carcinomas
cluster-w4	mAbs	Small cell lung carcinomas
cluster-5A	mAbs	Small cell lung carcinomas
cluster-6 (LeY)	mAbs	Small cell lung carcinomas
PLAP (placental alkaline phosphatase)	mAbs	Some seminomas Some ovarian; some non-small cell lung cancer
CA-125	mAbs	Lung, ovarian carcinoma
ESA (epithelial specific antigen)	mAbs	
CD 19, 22, 37	mAbs	B-cell lymphoma
250 kDa proteoglycan	mAbs	Melanoma
p55	mAbs	Breast cancer
TCR-IgH fusion	mAbs	Childhood T-cell leukaemia
Blood gp A antigen (in B or O individuals)	mAbs	Gastric and colon tumours

The binding moiety may be a monoclonal antibody. Monoclonal antibodies which will bind to many of these antigens are already known but in any case, with today's techniques in relation to monoclonal antibody technology, antibodies can be prepared to most antigens. The binding moiety may be a part of an antibody (for example a Fab fragment) or a synthetic antibody fragment (for example, ScFv). Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in *"Monoclonal Antibodies: A manual of techniques"*, H Zola (CRC Press, 1988) and in *"Monoclonal Hybridoma Antibodies: Techniques and Applications"*, J G R Hurrell (CRC Press, 1982).

Suitably prepared non-human antibodies can be "humanized" in known ways, for example by inserting the CDR regions of mouse antibodies into the framework of human antibodies.

The variable heavy (V_H) and variable light (V_L) domains of the antibody are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further confirmation was found by "humanization" of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent parental antibody (Morrison *et al* (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6851-6855).

That antigenic specificity is conferred by variable domains and is independent of the constant domains is known from experiments involving the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better *et al* (1988) *Science* **240**, 1041); Fv molecules (Skerra *et al* (1988) *Science* **240**, 1038); ScFv molecules where the V_H and V_L partner domains are

linked via a flexible oligopeptide (Bird *et al* (1988) *Science* **242**, 423; Huston *et al* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5879) and dAbs comprising isolated V domains (Ward *et al* (1989) *Nature* **341**, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) *Nature* **349**, 293-299.

By "ScFv molecules" we mean molecules wherein the V_H and V_L partner domains are linked via a flexible oligopeptide.

It may be advantageous to use antibody fragments, rather than whole antibodies. Effector functions of whole antibodies, such as complement binding, are removed. ScFv and dAb antibody fragments can be expressed as fusions with other polypeptides.

Minimal recognition units may be derived from the sequence of one or more of the complementary-determining regions (CDR) of the Fv fragment. Whole antibodies, and $F(ab')_2$ fragments are "bivalent". By "bivalent" we mean that the said antibodies and $F(ab')_2$ fragments have two antigen combining sites. In contrast, Fab, Fv, ScFv, dAb fragments and minimal recognition units are monovalent, having only one antigen combining sites.

In a further embodiment the binding moiety is at least part of a ligand of a target cell-specific cell-surface receptor.

It is preferred that the target cell-specific cell-surface receptor is the receptor for human gonadotrophin releasing hormone (GnRH). In this preferred embodiment the binding moiety is GnRH, and its binding specificity is for human cancer cells that express the GnRH receptors on

their surface. Examples of such human cancer cells are prostate, breast and endometrial cancer cells.

It is also preferred that the target cell-specific cell-surface receptor is the receptor for melanocyte-stimulating hormone (MSH) which is expressed in high number in melanoma cells. In this preferred embodiment the binding moiety is MSH, and its binding specificity is for melanoma cells.

It is also preferred that the target cell-specific cell-surface receptor is the receptor for somatostatin.

Of course, the receptors for GnRH, MSH and somatostatin may themselves be target cell-specific antigens and may be recognised by binding moieties which have the property of any one of a monoclonal antibody, a ScFv, a dAb or a minimal recognition unit. Thus, although the binding site on the target cell may be a cell-surface receptor it may also act as a target cell-specific cell-surface antigen for recognition by the binding moiety.

It will be appreciated by those skilled in the art that binding moieties which are polypeptides may be conveniently made using recombinant DNA techniques. The binding moiety may be fused to a protein on the surface of the virus or virus-like protein as disclosed below or they may be synthesised independently of the virus or virus-like particle, by expression from a suitable vector in a suitable host and then joined to the virus or virus-like particle as disclosed below.

Nucleic acid sequences encoding many of the targeting moieties are known, for example those for peptide hormones, growth factors, cytokines and the like and may readily be found by reference to publicly accessible

nucleotide sequence databases such as EMBL and GenBank. Once the nucleotide sequence is known it is obvious to the person skilled in the art how to make DNA encoding the chosen binding moiety using, for example, chemical DNA synthetic techniques or by using the polymerase chain reaction to amplify the required DNA from genomic DNA or from tissue-specific cDNA.

Many cDNAs encoding peptide hormones, growth factors, cytokines and the like, all of which may be useful as binding moieties, are generally available from, for example British Biotechnology Ltd, Oxford, UK.

It is preferred that when the virus or virus-like particle of the invention binds to its target cell it delivers its nucleic acid to the said target cell, that is the target cell is infected by the virus or virus-like particle. Target cells, especially cancer cells, that are infected in this manner by the virus or virus-like particle may express viral molecules on their surface and may be recognised by the immune system and destroyed. Of course, other cytotoxic functions of the virus may also kill the cell.

In one embodiment when the virus or virus-like particle is adenovirus, the E1B gene is substantially deleted or modified so that its gene product no longer interacts with the E1A protein. E1A protein stimulates apoptosis but normally its action is inhibited by E1B. Conveniently, the E1B gene is inactivated by insertion; preferably a cytotoxic gene, as defined below, is inserted at or near the E1B gene.

E1, E3 and a site upstream of E4 may be used as sites for insertion of foreign DNA sequences in the generation of recombinant adenoviruses for example see Berkner and Sharp (1984) *Nucl. Acids Res.* 12, 1925-1941; Chanda *et al* (1990) *Virology* 175, 535-547; Haj-Ahmad and Graham

(1986) *J. Virol.* **57**, 267-274; Saito *et al* (1985) *J. Virol.* **54**, 711-719; all incorporated herein by reference. Since the upper size limit for DNA molecules that can be packaged into adenovirus particles is approximately 105% of the wild-type genome only about 2 kb of extra DNA can be inserted without compensating deletions of viral DNA. Although E1 is essential for virus replication in cell culture, foreign DNA can be substituted for E1 sequences when the virus is grown in 293 cells which are transformed by Ad5 DNA and constitutively express E1 (Graham *et al* (1977) *J. Gen. Virol.* **36**, 59-72, incorporated herein by reference). Several vectors having 1.9 kb deleted from E3 of Ad5 have been constructed without interfering with virus replication in cell culture (reviewed by Graham and Prevec (1992) in "Vaccines: New Approaches to Immunological Problems" R.W. Ellis (Ed.), Butterworth-Heinemann, Boston, MA, pages 364-390, incorporated herein by reference). Such vectors allow for insertion of up to 4 kb of foreign DNA. Recombinant adenoviruses containing inserts in E3 replicate in all Ad-permissive cell lines and a number of adenovirus vectors containing E3 inserts have been shown to express foreign genes efficiently both *in vitro* and *in vivo* (Berkner (1988) *Biotechniques* **6**, 616-629; Chanda *et al* (1990) *Virology* **175**, 535-547; Dewar *et al* (1989) *J. Virol.* **63**, 129-136; Graham (1990) *Trends Biotechnol.* **8**, 85-87; Graham and Prevec (1992) in "Vaccines: New Approaches to Immunological Problems" R.W. Ellis (Ed.), Butterworth-Heinemann, Boston, MA, pages 364-390; Johnson *et al* (1988) *Virology* **164**, 1-14; Lubeck *et al* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6763-6767; McDermott *et al* (1989) *Virology* **169**, 244-247; Morin *et al* (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4626-4630; Prevec *et al* (1989) *J. Gen. Virol.* **70**, 429-434; Prevec *et al* (1990) *J. Inf. Dis.* **161**, 27-30; Schneider *et al* (1989) *J. Gen. Virol.* **70**, 417-427; Vernon *et al* (1991) *J. Gen. Virol.* **72**, 1243-1251; Yuasa *et al* (1991) *J. Gen. Virol.* **72**, 1927-1934) all incorporated herein by reference.

Substantially replication-defective adenoviruses may be made by creating a deficiency of the E1A protein. Suitably this is achieved by deleting the E1A gene or by making mutations within the E1A gene that prevent expression of the E1A protein. Examples of suitable mutations are deletions within the E1A coding region; nonsense mutations; and frameshift mutations.

In further preference, the virus or virus-like particle is modified further to contain a gene suitable for gene therapy.

In one embodiment, the gene encodes a molecule having a directly or indirectly cytotoxic function. By "directly or indirectly" cytotoxic, we mean that the molecule encoded by the gene may itself be toxic (for example ricin; tumour necrosis factor; interleukin-2; interferon-gamma; ribonuclease; deoxyribonuclease; Pseudomonas exotoxin A) or it may be metabolised to form a toxic product, or it may act on something else to form a toxic product. The sequence of ricin cDNA is disclosed in Lamb *et al* (1985) *Eur. J. Biochem.* 148, 265-270 incorporated herein by reference.

For example, it would be desirable to target a DNA sequence encoding an enzyme using the virus or virus-like particle of the invention, the enzyme being one that converts a relatively non-toxic prodrug to a toxic drug. The enzyme cytosine deaminase converts 5-fluorocytosine (5FC) to 5-fluorouracil (5FU) (Mullen *et al* (1922) *PNAS* 89, 33); the herpes simplex enzyme thymidine kinase sensitises cells to treatment with the antiviral agent ganciclovir (GCV) or aciclovir (Moolten (1986) *Cancer Res.* **46**, 5276; Ezzedine *et al* (1991) *New Biol* 3, 608). The cytosine deaminase of any organism, for example *E. coli* or *Saccharomyces cerevisiae,* may be used.

Thus, in a preferred embodiment of the invention, the gene encodes a cytosine deaminase and the patient is concomitantly given 5FC. By "concomitantly", we mean that the 5FC is administered at such a time, in relation to the transformation of the tumour cells, that 5FC is converted into 5FU in the target cells by the cytosine deaminase expressed from the said gene. A dosage of approximately 0.001 to 100.0 mg 5FC/kg body weight/day, preferably 0.1 to 10.0 mg/kg/day is suitable.

Components, such as 5FC, which are converted from a relatively non-toxic form into a cytotoxic form by the action of an enzyme are termed "pro-drugs".

Other examples of pro-drug/enzyme combinations include those disclosed by Bagshawe *et al* (WO 88/07378), namely various alkylating agents and the *Pseudomonas* spp. CPG2 enzyme, and those disclosed by Epenetos & Rowlinson-Busza (WO 91/11201), namely cyanogenic pro-drugs (for example amygdalin) and plant-derived β-glucosidases.

Enzymes that are useful in this embodiment of the invention include, but are not limited to, alkaline phosphatase useful for converting phosphate-containing prodrugs into free drugs; arylsulfatase useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as serratia protease, thermolysin, subtilisin, carboxypeptidases and cathepsins (such as cathepsins B and L), that are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β-galactosidase and neuraminidase useful for converting glycosylated prodrugs into free drugs; β-lactamase useful for converting drugs

derivatized with β-lactams into free drugs; and penicillin amidases, such as penicillin V amidase or penicillin G amidase, useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. Alternatively, antibodies with enzymatic activity, also known in the art as abzymes, can be used to convert the prodrugs of the invention into free active drugs [see, e.g. R J Massey, *Nature*, 328, pp. 457-458 (1987)].

Similarly, the prodrugs of this invention include, but are not limited to, the above-listed prodrugs, e.g., phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β-lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorouridine prodrugs which can be converted by the enzyme of the conjugate into the more active, cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, etoposide, teniposide, adriamycin, daunomycin, carminomycin, aminopterin, dactinomycin, mitomycins, cis-platinum and cis-platinum analogues, bleomycins, esperamicins [see U.S. Pat. No. 4,675,187], 5-fluorouracil, melphalan and other related nitrogen mustards.

In a further embodiment the gene delivered to the target cell encodes a ribozyme capable of cleaving targeted RNA or DNA. The targeted RNA or DNA to be cleaved may be RNA or DNA which is essential to the function of the cell and cleavage thereof results in cell death or the RNA or DNA to be cleaved may be RNA or DNA which encodes an undesirable protein, for example an oncogene product, and cleavage of

this RNA or DNA may prevent the cell from becoming cancerous.

Ribozymes which may be encoded in the genomes of the viruses or virus-like particles herein disclosed are described in Cech and Herschlag "Site-specific cleavage of single stranded DNA" US 5,180,818; Altman *et al* "Cleavage of targeted RNA by RNAse P" US 5,168,053, Cantin *et al* "Ribozyme cleavage of HIV-1 RNA" US 5,149,796; Cech *et al* "RNA ribozyme restriction endoribonucleases and methods", US 5,116,742; Been *et al* "RNA ribozyme polymerases, dephosphorylases, restriction endonucleases and methods, US 5,093,246; and Been *et al* "RNA ribozyme polymerases, dephosphorylases, restriction endoribonucleases and methods; cleaves single-stranded RNA at specific site by transesterification", US 4,987,071, all incorporated herein by reference.

In a still further embodiment the gene delivered to the target cell encodes an antisense RNA.

By "antisense RNA" we mean an RNA molecule which hybridises to, and interferes with the expression from a mRNA molecule encoding a protein or to another RNA molecule within the cell such as pre-mRNA or tRNA or rRNA, or hybridises to, and interferes with the expression from a gene.

Conveniently, a gene expressing an antisense RNA may be constructed by inserting a coding sequence encoding a protein adjacent a promoter in the appropriate orientation such that the RNA complementary to mRNA. Suitably, the antisense RNA blocks expression of undesirable polypeptides such as oncogenes, for example *ras, bcl, src* or tumour suppressor genes such as p53 and *Rb*.

It will be appreciated that it may be sufficient to reduce expression of the

undesirable polypeptide rather than abolish the expression.

It will be further appreciated that DNA sequences suitable for expressing as antisense RNA may be readily derived from publicly accessible databases such as GenBank and EMBL.

In another embodiment of the invention, the gene replaces the function of a defective gene in the target cell.

There are several thousand inherited genetic diseases of mammals, including humans, that are caused by defective genes. Examples of such genetic diseases include cystic fibrosis, where there is known to be a mutation in the CFTR gene; Duchenne muscular dystrophy, where there is known to be a mutation in the dystrophin gene; sickle cell disease, where there is known to be a mutation in the HbA gene. Many types of cancer are caused by defective genes, especially protooncogenes, and tumour-suppressor genes that have undergone mutation.

Thus, it is preferred that the virus or virus-like particle of the invention, which may be useful in the treatment of cystic fibrosis, contains a functional CFTR gene to replace the function of the defective CFTR gene. Similarly, it is preferred that the virus or virus-like particle of the invention, which may be useful in the treatment of cancer, contains a functional protooncogene, or tumour-suppressor gene to replace the function of the defective protooncogene or tumour-suppressor gene.

Examples of protooncogenes are *ras, src, bcl* and so on; examples of tumour-suppressor genes are p53 and *Rb*.

By "gene" we mean a nucleic acid coding sequence that may contain

introns, or fragment thereof, or cDNA, or fragment thereof.

It will be appreciated that the gene will be introduced into a convenient place within the genome of the virus or virus-like particle and will contain a promoter and/or enhancer element to drive its expression.

It is preferred if the promoter and/or enhancer is selective for the cells to be targeted. Some examples of tissue or tumour specific promoters are given below but new ones are being discovered all of the time which will be useful in this embodiment of the invention.

The tyrosinase and TRP-1 genes both encode proteins which play key roles in the synthesis of the pigment melanin, a specific product of melanocytic cells. The 5′ ends of the tyrosinase and tyrosinase-related protein (TRP-1) genes confer tissue specificity of expression on genes cloned downstream of these promoter elements.

The 5′ sequences of these genes are described in Bradl, M. *et al* (1991) *Proc. Natl. Acad. Sci. USA* **88**, 164-168 and Jackson, I.J. *et al* (1991) *Nucleic Acids Res.* **19**, 3799-3804.

Prostate-specific antigen (PSA) is one of the major protein constituents of the human prostate secretion. It has become a useful marker for the detection and monitoring of prostate cancer. The gene encoding PSA and its promoter region which directs the prostate-specific expression of PSA have been described (Lundwall (1989) *Biochem. Biophys. Res. Comm.* **161**, 1151-1159; Riegman *et al* (1989) *Biochem. Biophys. Res. Comm.* **159**, 95-102; Brawer (1991) *Acta Oncol.* **30**, 161-168).

Carcinoembryonic antigen (CEA) is a widely used tumour marker,

especially in the surveillance of colonic cancer patients. Although CEA is also present in some normal tissues, it is apparently expressed at higher levels in tumorous tissues than in corresponding normal tissues. The complete gene encoding CEA has been cloned and its promoter region analysed. A CEA gene promoter construct, containing approximately 400 nucleotides upstream from the translational start, showed nine times higher activity in the adenocarcinoma cell line SW303, compared with the HeLa cell line. This indicates that *cis*-acting sequences which convey cell type specific expression are contained within this region (Schrewe *et al* (1990) *Mol. Cell. Biol.* **10**, 2738-2748).

The c-*erb*B-2 gene and promoter have been characterised previously and the gene product has been shown to be over-expressed in tumour cell lines (Kraus *et al* (1987) *EMBO J.* **6**, 605-610).

The mucin gene, MUC1, contains 5′ flanking sequences which are able to direct expression selectively in breast and pancreatic cell lines, but not in non-epithelial cell lines as taught in WO 91/09867.

The binding moiety allowing the virus or virus-like particle to bind to a target cell may be a polypeptide or oligosaccharide or lipid or any other molecule capable of binding specifically to the target cell.

It is preferred that the binding moiety is a polypeptide.

The molecule on the surface of the virus or virus-like particle to which the binding moiety is joined may be a polypeptide, oligosaccharide or lipid or any other molecule in the virus or virus-like particle coat. It is preferred that the molecule is a polypeptide.

If the binding moiety and the molecule on the surface of the virus or virus-like particle are both polypeptides then they may be linked together by any of the conventional ways of cross-linking polypeptides, such as those generally described in O'Sullivan *et al Anal. Biochem.* (1979) **100**, 100-108. For example, the binding moiety may be enriched with thiol groups and the molecule on the surface of the virus or virus-like particle reacted with a bifunctional agent capable of reacting with those thiol groups, for example the N-hydroxysuccinimide ester of iodoacetic acid (NHIA) or N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP). Amide and thioether bonds, for example achieved with m-maleimidobenzoyl-N-hydroxysuccinimide ester, are generally more stable *in vivo* than disulphide bonds.

Other chemical procedures may be useful in joining oligosaccharide and lipids to other oligosaccharides, lipids or polypeptides.

It is preferred that the binding moiety and the molecule on the surface of the virus or virus-like particle are both polypeptides that may be produced as a fusion by the techniques of genetic engineering. The use of genetic engineering allows for the precise control over the fusion of such polypeptides.

Thus a further embodiment of the invention is a nucleotide sequence encoding the fusion of the binding moiety and the protein on the surface of the virus or virus-like particle.

The nucleotide sequence encoding the fusion of the binding moiety and the protein on the surface of the virus or virus-like particle is preferably made by an alteration of the viral genome.

The nucleotide sequence may be synthesised *de novo* using solid phase phosphoramidite chemistry, but it is more usual for the nucleotide sequence to be constructed from two parts, the first encoding the binding moiety and the second the protein on the surface of the virus or virus-like particle. The two parts may be derived from their respective genes by restriction endonuclease digestion or by other methods known by those skilled in the art such as the polymerase chain reaction.

A variety of methods have been developed to operatively link two nucleotide sequences via complementary cohesive termini. For instance, synthetic linkers containing one or more restriction sites provide a method of joining the two DNA segment together. Each DNA segment, generated by endonuclease restriction digestion, is treated with bacteriophage T4 DNA polymerase of *E. coli* DNA polymerase I, enzymes that remove protruding, 3'-single-stranded termini with their 3'-5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and lifted to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CN, USA.

A desirable way to generate the DNA encoding the fusion polypeptide of the invention is to use the polymerase chain reaction as disclosed by Saiki *et al* (1988) *Science* 239, 487-491.

In this method each of the DNA molecules encoding the two polypeptides to be fused are enzymatically amplified using two specific oligonucleotide primers which themselves become incorporated into the amplified DNA. The said specific primers may contain restriction endonuclease recognition sites which may then be used to join the said two DNA molecules using T4 DNA ligase as disclosed.

A particular feature of one aspect of the present invention is the modification of the virus or virus-like particle of the invention so that it no longer binds its host cell and so that it binds the target cell by virtue of its binding moiety.

The host-cell receptor of adenovirus may be the penton fibre and that of influenza virus may be the haemagglutinin receptor.

These receptors may be modified by the insertion or deletion or substitution of amino acid residues that disrupt their host-cell binding function. It is preferred that the binding moiety for the target cell is joined to the host-cell receptor in such a manner that the binding moiety is capable of binding the target cell, the host-cell receptor is unable to bind to the host cell and therefore the binding specificity of the virus or virus-like particle is modified. A further preference is that the portion of the host-cell receptor that is exposed on the surface of the virus or virus-like particle is replaced by the binding moiety, and that the portion of the host-cell receptor which promotes the uptake of viral DNA by the target cell is retained. Suitably, the binding moiety is joined directly or

indirectly to the host-cell receptor by a spacer group.

Examples of spacer groups are polypeptide sequences of between 4 and 1000 amino acid residues.

Thus, in one embodiment of the invention the gene encoding the penton fibre in adenovirus is modified in such a way that the DNA encoding the surface-exposed portion is replaced by a DNA fragment encoding a ScFv, the ScFv being derived from an antibody which binds to a target cell surface antigen.

Potential fusion sites within the penton fibre have been identified.

The adenovirus fibre is a trimer composed of three protomers. The amino terminal end (40 amino acids or so) of each participates in the formation of a tail that is closely associated with the penton (as opposed to the hexon) subunit of the capsid. High amino acid conservation is maintained between the different characterised serotypes.

Middle portions of each protomer form the shaft of the protein. This shaft is of variable length, depending upon serotype, and is composed of repeating units of 15 amino acids (for examples, serotypes have been identified with 6, 15 and 21 repeat units). These repeating units are not duplicates: rather than strict conservation of amino acid structure, there is a general conservation of relative hydrophobicity. Some serotypes, for example, 40 and 41, have shafts composed of different length fibre proteins. This suggests a certain flexibility in structural constraints.

The carboxy-terminal ends (some 200 amino acids) associate to form a knob that is held erect a great distance (in molecular terms) from the

capsid.

Whilst the cellular receptor(s) and mechanisms of docking have not been firmly identified and elucidated, we propose that the most likely candidate structure for cell binding is the knob. Thus, in one embodiment the whole knob of the penton fibre has been replaced with single chain antibody (ScFv) domains. The triplex structure implies that each fibre will thus end in three ScFvs. Additionally, the ScFv regions can be replaced with CDRs, or by non-antibody derived peptides, of known specificity or other molecules that are capable of interacting specifically with the target cell.

Suitable fusion sites are therefore at the native junction between shaft and knob domains, or (should the DNA sequence prove to be more amenable) at any junction between repetitive units of the shaft. Preferably, the minimum shaft length is not reduced beyond the smallest size naturally identified. There are thus at least 15 potential sites at which fusion could be contemplated.

Although it is preferred that the binding moiety forms the end of the fibre thereby replacing the knob, the binding moiety may also be fused within the penton fibre sequence but still display its binding surfaces and bind to the target cell.

Suitably, the binding moiety may be fused to the knob and extend externally to the knob structure.

In a further embodiment influenza virus haemmaglutinin is modified to incorporate a binding moiety. Influenza virus has seven or eight (depending on serotype) genetic segments, all negative strand RNA. Suitably, a cDNA from the whole segment encoding haemmagglutinin is

constructed and modified by adding a promoter firing *backwards* across this segment so that negative strand RNA is made. Genetic fusions with a suitable binding molecule, as disclosed above, are made using standard recombinant DNA methods and a suitable cell line is stably transfected with this gene construct. Infection of this transfected cell line with influenza virus and selection of reassorted genomes containing the new haemmagglutinin by infection of a normally resistant cell line that expresses a marker that can only be recognised by the new haemmagglutinin yields the desired virus comprising modified cell-binding specificity.

A further aspect of the invention provides a method of producing in cell culture a virus or virus-like particle and then joining the binding moiety, as defined above, to the virus or virus-like particle.

A further aspect of the invention provides a method of producing in cell culture a virus or virus-like particle which has been genetically modified to express a binding moiety on its surface. The virus or virus-like particle is grown in its host prior to modification, but once the modification that alters the binding specificity is made, the virus or virus-like particle is grown in the target cell. Thus, for example in the case where the binding moiety recognises a breast tumour cell antigen, the virus or virus-like particle is grown in breast tumour cell culture.

The virus or virus-like particles of the invention are administered in any suitable way, usually parenterally, for example intravenously, intraperitoneally or intravesically, in standard sterile, non-pyrogenic formulations of diluents and carriers, for example isotonic saline (when administered intravenously).

A further aspect of the invention provides a method of delivery of the virus or virus-like particle which contains a gene encoding a molecule having an indirectly cytotoxic function.

Suitably, the indirectly cytotoxic function is an enzyme that converts a prodrug to a toxic drug. With such a virus or virus-like particle, once the virus or virus-like particle has bound to the target cells, delivered its nucleic acid to the cells, and expressed the indirectly cytotoxic functions, which typically takes a day or so, the pro-drug is administered. The timing between administration of the virus or virus-like particle and the pro-drug may be optimised in a non-inventive way.

The dosage of the pro-drug will be chosen by the physician according to the usual criteria. The dosage of the virus or virus-like particle will similarly be chosen according to normal criteria, and in the case of tumour treatment, particularly with reference to the type, stage and location of tumour and the weight of the patient. The duration of treatment will depend in part upon the rapidity and extent of any immune reaction to the virus or virus-like particle.

Some of the viruses or virus-like particles either in themselves, or together with an appropriate pro-drug, are in principle suitable for the destruction of cells in any tumour or other defined class of cells selectively exhibiting a recognisable (surface) entity. Examples of types of cancer that may be treated using the viruses or virus-like particles are cancer of the breast, prostate, colon, rectum, ovary, testicle and brain. The compounds are principally intended for human use but could be used for treating other mammals including dogs, cats, cattle, horses, pigs and sheep.

The invention will now be described in detail with reference to the

following Figures and Examples in which:

Figure 1 shows (a) an unmodified (i.e. "naive") virus or virus-like particle able to bind to and infect its host cell but not a non-host cell, such as a target cell; and (b) a virus or virus-like particle with a modified binding specificity does not bind and infect its host cell but binds and infects a target cell; and (c) a virus or virus-like particle as in (b) modified further to contain a gene for gene therapy or cancer treatment.

Figure 2 shows (a) unmodified (naive) adenovirus; (b) adenovirus modified so that its penton fibres, which recognise the host cell, are replaced in part by antibody fragments which recognise the target cell; and (c) adenovirus as in (b) with further genetic material added to the viral DNA for gene therapy of cancer.

Figure 3 shows (a) influenza virus and (b) genetically-modified influenza virus wherein at least part of the haemagglutinin binding site is replaced by an antibody with anti-cancer cell binding activity.

Figure 4 shows (a) a retrovirus virus; and (b) as in (a) except the retrovirus has been modified further to express on its surface an anticancer cell-binding antibody fragment or an anticancer cell-binding peptide.

Figure 5 is a diagrammatic representation of a penton fibre indicating potential fusion sites within the fibre.

Figure 6 shows fusions between the DNA encoding the Ad5 fibre and an ScFv.

Figure 7 shows sequences of oligonucleotides used for amplifying the

ScFv. All oligonucleotides are presented 5′ to 3′, the reverse complement of FOR primers are shown and derived amino acid sequences are shown where relevant.

Figure 8 shows the construction of plasmid pRAS117.

Figure 9 shows the nucleotide and derived amino acid sequence between the *Hind*III and *Eco*RI sites of pRAS117.

Figure 10 shows a map of plasmid pRAS117.

Figure 11 is a diagrammatic representation of the construction of plasmid pRAS118.

Figure 12 shows the sequences of oligonucleotides for amplifying Ad5 fibre DNA fragments. All oligonucleotides are presented 5′ → 3′. The reverse complements of FOR primers are shown. Derived amino acid sequences are shown where relevant.

Figure 13 shows the nucleotide sequence and deduced amino acid sequence between the *Hind*III site and *Eco*RI site of pRAS111.

Figure 14 gives a diagrammatic representation of constructing adenovirus carrying a cytotoxic gene.

Figure 15 gives the nucleotide and amino acid sequences of mouse and humanised HMFG1 variable regions.

Example 1: Fusion sites within the adenovirus Ad5 fibre for binding moieties including single chain Fv (ScFv)

The Ad5 DNA sequence co-ordinates used here are taken from:

ADRCOMPGE_1: residues 1 to 32760

and ADRCOMPGE_2: residues 32761-35935

These can be accessed by using program SEQ on the Intelligenetics database.

The sequence of Ad5 fibre can also be found in Chroboczek, J. and Jacrot, B. (1987) "The sequence of adenovirus fiber: Similarities and differences between serotypes 2 and 5" *Virology* **161**, 549-554 and is available from the EMBL Database, Heidelberg, Germany under accession name ADEFIB.

Fusion sequences between the shaft and the ScFv are shown in Fig. 6. The fusion sites are at the junctions of the repetitive units of the shaft. Shaft sequences are shown in normal typescript; ScFv sequences are shown in italics. The DNA sequence between the *Pst*I and *Xho*I sites is unique to the ScFv used.

Fusion A is at the end of the first repetitive unit of the shaft (co-ordinates 31218-9), fusion B at the end of the second (31266-7), fusion C at the third (31323-4), fusion D at the fourth (31368-9), fusion E at the fifth (31413-4), fusion F at the sixth (31458-9), fusion G at the seventh (31503-4), fusion H at the eighth (31551-2), fusion I at the ninth (31596-7), fusion J at the tenth (31641-2), fusion K at the eleventh (31692-3), fusion L at the twelfth (31737-8), fusion M at the thirteenth (31787-8), fusion N at the fourteenth (31836-7), fusion O at the fifteenth (31884-5), fusion P

at the sixteenth (31929-30), fusion Q at the seventeenth (31995-6), fusion R at the eighteenth (32040-1), fusion S at the nineteenth (32103-4), fusion T at the twentieth (32151-2), fusion U at the twenty-first (32199-200), and fusion V is at the end of the twenty-second repetitive unit of the shaft (32244-5), the junction between shaft and knob.

Example 2: Preparation of adenovirus expressing an ScFv on its surface

The genetically modified fibre is introduced into the Ad5 genome by: (a) replacing the fibre gene of plasmid pE4 with the modified fibre by standard recombinant DNA technology and (b) reconstituting the virus by recombination.

pE4 is a plasmid containing the right hand half of the Ad5 genome, and which has served as the source of the Ad5 fibre gene that we have used. It was provided by Dr Keith Leppard, Biological Sciences, University of Warwick, Coventry, CV4 7AL who has supplied details of its structure. If it is introduced into mammalian cells that contain the remainder of the Ad5 genome, then it is possible to obtain recombinants containing the modification. Most human cell lines can be used for the recombination but HeLa cells are preferred.

The plasmid pE4 is readily made in the following way. A derivative of pBR322 is made by digesting with *Bst*N1 and rejoining using *Xho*I linkers such that the *Bst*N1 fragment corresponding to positions 1442-2502 in the pBR322 sequence is removed. DNA from the adenovirus Ad5 strain 309 described by Jones & Shenk (1979) *Cell* 17, 683-689 is isolated and deproteinated. This DNA is then ligated to *Cla*I linkers and cut with *Eco*RI and *Cla*I. The *Cla*I-*Eco*RI fragment corresponding to the region

of 76% of the Ad5 genome to the right hand end is isolated and cloned into the *Eco*RI-*Cla*I sites of the above-mentioned pBR322 derivative to form pE4.

Adenovirus Type 5 and HeLa cells are available from the American Type Culture Collection, 12301 Packlawn Drive, Rockville, MD 20852-1776, USA under accession numbers ATCC VR-5 and ATCC CCL-2.

Construction of plasmid pRAS117

Oligonucleotide primers LEADHBACK and LEADbFOR (Figure 7) were used for PCR-mediated amplification of the DNA segment extending from the *Hin*dIII site of plasmid pRAS111, over the Shine-Dalgarno sequence and the *pel*B leader sequence to the *Pst*I site in the ScFv. LEADbFOR directs the incorporation of a *Bgl*II site immediately after the *pel*B leader sequence. DNA (100 ng) from plasmid pRAS111 was subjected to 24 rounds of amplification, (94°C, 1 min; 65°C, 1.5 min and 72°C, 2 min) in a 50 μl reaction volume containing 25 pmol of each primer, 250 mM of each dNTP, 67 mM Tris-HCl (pH 8.8), 17 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 200 mg.ml^{-1} gelatine and 5 units of *Thermus aquaticus* (Taq) polymerase (Cetus) overlaid with 25 μl paraffin oil. After the reaction, oil was removed by extraction with 500 μl chloroform. The sample was loaded on a 2% agarose gel, and the amplified fragment was electrophoresed on to a piece of NA45 paper (Schleicher and Schuell). Bound DNA was subsequently eluted by immersion in 400 μl 1M NaCl made in TE (10 mM Tris-HCl, pH 7.5, 1 mM EDTA) for 30 min at 70°C. To this was added 800 μl ethanol, and after incubation (2 h, -20°C) the DNA was collected by centrifugation. The pellet was taken up in 50 μl T/E.

One fifth (10 μl) of the purified amplified fragment was cut with the restriction enzymes *Hind*III and *Pst*I, in a total volume of 20 μl 50 mM Tris-HCl, pH 7.5, 10 MgC12, 100 mM NaCl, 1 mM dithioerythreitol containing 10 units of each enzyme. After incubation (1 h, 37°C) the reaction was stopped by incubation at 70°C for 15 minutes.

The trimmed amplified fragment was cloned between the *Hind*III and *Pst*I sites of pUC8, to generate plasmid pRAS117.

Plasmid pUC8 (1 μg) was cut with *Hind*III and *Pst*I, in a total volume of 20 μl 50 mM Tris-HCl, pH 7.5, 10 $MgCl_2$, 100 mM NaCl, 1 mM dithioerythreitol containing 10 units of each enzyme. After incubation (1 h, 37°C) the reaction was stopped by incubation at 70°C for 15 minutes.

The ligation reaction contained 1.5 μl of pUC8/*Hind*III, *Pst*I and 3 μl of the amplified leader/*Hind*III, *Pst*I in a total volume of 15 μl containing 70 mM Tris-HCl pH 7.5, 7 mM $MgCl_2$, 0.7 mM rATP, 4 mM dithiothreitol, 0.5 $mg.ml^{-1}$ BSA and 10 units of T4 DNA ligase. After incubation (2 h, at room temperature), the reaction was stopped by the addition of 1 μl 500 mM EDTA, pH 8.0 and 14 μl H_2O.

This ligation mix was used to transform *E. coli*.

An aliquot (5 μl) of this ligation mix was used to transform a 200 μl aliquot of commercially available competent *E. coli* K12 DH5*8,1*αF (Life Sciences Inc). After incubation (30 min, 0°C), heat shock (2 min, 42°C), addition of 800 μl L-broth and recovery (37°C, 1 h), cells (100 μl) were spread on L-agar plates containing 100 $\mu g.ml^{-1}$ ampicillin containing 50 mM IPTG (isopropyl-β-D-galactopyranoside) and 100 $\mu g.ml^{-1}$ X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside). Cells were grown

overnight at 37°C, and individual colonies were transferred to fresh L-agar/ampicillin plates. After 6 h growth, colonies were used to inoculate 5 ml aliquots of L-broth containing 100 $\mu g.ml^{-1}$ ampicillin. These cells were grown overnight with shaking at 37°C, and used as a source of plasmid DNA.

These cells were used as a source of plasmid DNA.

Harvested cells were suspended in 360 μl of SET (50 mM sucrose, 10 mM EDTA, 100 mM Tris-HCl, pH 7.5) containing 2 $mg.ml^{-1}$ hen egg lysozyme, transferred to a 1.5 ml microfuge tube, and diluted by addition of 300 μl 10% Triton X-100. After floating on boiling water for 2 min and cooling for a further minute in ice/water, denatured cell debris was removed by centrifugation (14,000 x g, 20 min) in a microcentrifuge. The majority of the soluble remaining proteins were removed by addition of 300 μl 7.5 M ammonium acetate and centrifugation (14,000 x g, 10 min). Nucleic acids were precipitated by addition of 720 μl cold (-20°C) isopropanol and centrifugation (14,000 x g, 10 min). After rinsing the pellets with ethanol and drying, DNA was solubilised in 60 μl TE containing 170 $\mu g.ml^{-1}$RNase A.

Restriction enzyme digestions on 5 μl aliquots, using the enzymes *Hin*dIII and *Bgl*II identified which of these plasmids were pRAS117. The construction scheme is shown in Fig. 8. The nucleotide and derived amino acid sequences between the *Hin*dIII and *Eco*RI sites of pRAS117 are shown in Fig. 9. A map of plasmid pRAS117 is provided in Fig. 10.

The nucleotide sequence of the relevant portion of pRAS111, between the *Hin*dIII site and *Eco*RI, site is given in Figure 13.

FIBRE3FOR, FIBRE6FOR, FIBRE9FOR, FIBRE12FOR, FIBRE15FOR, FIBRE18FOR, FIBRE21FOR or FIBRE22FOR. Oligonucleotide sequences can be found in Fig. 12.

TAILdBACK directs the incorporation of a *BglII* site at the base of the fibre, and the FIBREnFOR series primers direct the incorporation of a *PstI* site at the junctions of repetitive shaft units 3-4 (FIBRE3FOR), 6-7 (FIBRE6FOR), 9-10 (FIBRE9FOR), 12-13 (FIBRE12FOR), 15-16 (FIBRE15FOR), 18-19 (FIBRE18FOR), 21-22 (FIBRE21FOR), between unit 22 and the knob (FIBRE22FOR) or at the end of the knob sequence (FIBREPFOR).

Fusion of fibre and ScFv

The amplified segments of fibre are trimmed with *BglII* and *PstI* and ligated between the *BglII* and *PstI* sites of plasmid pRAS118. This gives a range of fusions under the transcriptional control of the T7 promoter. Colonies are recovered after transformation of a suitable *E. coli* strain, such as DH5, which does not permit expression of the fusions.

Screening

Colonies containing candidates for fusion are identified by restriction digestion of their plasmid DNAs. These candidate DNAs are used to transform a suitable *E. coli* strain, such as BL21 (DE3), that contains a chromosomal insertion of T7 polymerase under *lac* control. In these cells, induction of expression of T7 polymerase using the gratuitous inducer IPTG causes expression of the fusion proteins. Soluble NIP-reactive material is identified in colonies with correctly assembled fusions. The DNA of these is identified and the NIP-reactive ScFv derived from

pRAS111 are replaced with a cell-binding ScFv.

Replacing the fibre:ScFv in plasmid pE4

There is a *Hin*dIII site approximately half-way along the fibre gene. Fusions with long fibres also contain this *Hin*dIII site. The fusion is introduced at this site.

Recombination *in vivo* of plasmid pE4-ScFv with the adenovirus genome

To obtain virus particles expressing the ScFv on the penton fibre suitable cells, such as 293 cells, are cotransfected with plasmid pE4-ScFv and plasmid pFG173 as described in Mittal *et al* (1993) *Virus Res.* 28, 67-90, incorporated herein by reference. Since neither pFG173 nor pE4-ScFv individually is able to generate virus progeny, on transfection of 293 cells viable virus progeny are only produced by *in vivo* recombination between these two plasmids resulting in rescue of the penton fibre-ScFv fusion into the Ad5 genome.

293 cells are human transformed primary embryonal cells available from the ATCC under accession number ATCC CRL 1573.

The adenovirus particles made in this way express a NIP-binding ScFv on their surface. Such particles are useful in a two-step targeting approach wherein a target-cell specific binding moiety, such as those identified in Tables 1 and 2, are joined to NIP molecule and targeted to a cell. Once they have localized to the target cell within the patient, the adenovirus displaying NIP-binding ScFv is administered to the patient and binds to the NIP.

Example 3: Insertion of a cytotoxic gene into the E3 region of adenovirus Ad5

In preparation for rescue of the cytotoxic gene into the E3 region of Ad5, the cytotoxic coding sequences were first inserted into a cassette containing the SV40 early promoter and poly A addition sequences to give plasmid pTOX as shown in Figure 14.

To obtain virus with the cytotoxic gene and SV40 regulatory sequences in the E3 region, 293 cells are cotransfected with plasmids pTOX and pFG173 (Fig 14). The plasmid pFG173 is constructed from pFG140, an infectious plasmid containing the Ad5 d1309 genome in circular form by inserting a kan^r gene at the *Eco*RI site as 75.9 m.u. as described in Grahm (1984) *EMBO J.* **3**, 2917-2922 and Mitall *et al* (1993) *Virus Res.* **28**, 67-90.

Since neither pFG173 nor pTOX individually is able to generate infectious virus progeny, on transfection of 293 cells viable virus progeny are only produced by *in vivo* recombination between these two plasmids resulting in rescue of the E3 insert into the Ad5 genome.

Viral plaques obtained after cotransfection are isolated and expanded in 293 cells and viral DNA was analyzed on an agarose gel after digestion with *Hin*dIII. The structure of the desired Ad5-cytotoxic gene recombinant is verified by the presence of diagnostic fragments. One recombinant is plaque purified and used for further study.

Legend to Figure 14

The plasmid pFG173 contains the entire Ad5 genome, except for a 3.2 kb

sequence spontaneously deleted between m.u. 75.9-84.9. Plasmids pTOX and pFG173 were used for cotransfection of 293 cells to rescue, by *in vivo* recombination, the cytotoxic gene flanked by SV40 regulatory sequences in the E3 region of Ad5. The resulting Ad5-cytotoxic gene recombinant was named Ad5-TOX. The relative positions of *Hin*dIII and *Xba*I restriction sites of the Ad5-TOX genome are shown. The position and orientation of the SV40 promoter, the cytotoxic gene, and the SV40 polyadenylation signal are shown below. Solid bars: luciferase gene; open bars: SV40 promoter and SV40 polyadenylation signal; hatched bars: amp^r and kan^r genes.

The cytotoxic gene is the cDNA for thymidine kinase.

Other cytotoxic genes are inserted into the E3 region of Ad5 in an analogous manner.

Example 4: Single chain Fv from the mouse monoclonal antibody HMFG1 and humanised monoclonal antibody Hu HMFG1

The nucleotide sequences encoding the V_H heavy chains and $V\kappa$ light chains of HMFG1 and Hu HMFG1 are shown in Figure 15 and are given in Verhoeyen *et al* (1993) *Immunology* 78, 364-370, incorporated herein by reference.

Legend to Figure 15

Nucleotide and amino acid sequences of mouse and reshaped HMFG1 variable regions. (a) Heavy chain variable region sequences for mouse and reshaped HMFG1 (Mo V_H-HMFG1 and Hu V_H-HMFG1); (b) mouse and reshaped light chain variable regions respectively (Mo V_κ-HMFG1 and

Hu V_κ-HMFG1). Amino acids numbering and definition of the CDR and framework regions are from Kabat *et al* (1987) *Sequences of Proteins of Immunological Interest*, Edn 4, US Dept of Health and Human Services Public Health Service, NIH, Bethesda, MD 20892, USA.

The methods described by Bird *et al* (1988) *Science* **242**, 423 or Huston *et al* (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5879 are applied to the nucleotide sequences described in Figure 15 to generate genes encoding ScFv for HMFG1 and ScFv for Hu HMFG1. These genes are fused individually into the adenovirus penton fibre gene as described in Examples 1 and 2.

The amino acid sequences of the V_H and V_L chains of H17E2 are disclosed in "Monoclonal antibodies - applications in clinical oncology", pages 37-43, 1991, A.A. Epenetos, ed., Chapman & Hall, UK.

Nucleotide sequences encoding the V_H and V_L chains are readily derived from the amino acid sequence using the genetic code and an ScFv can be made from the sequences using the methods of Bird *et al* or Huston *et al* as described above.

Key to Sequence Listing

Name	SEQ ID No.	
	Nucleotide Sequence	Polypeptide Sequence

Fusion A	1	2
Fusion B	3	4
Fusion C	5	6
Fusion D	7	8
Fusion E	9	10
Fusion F	11	12
Fusion G	13	14
Fusion H	15	16
Fusion I	17	18
Fusion J	19	20
Fusion K	21	22
Fusion L	23	24
Fusion M	25	26
Fusion N	27	28
Fusion O	29	30
Fusion P	31	32
Fusion Q	33	34
Fusion R	35	36
Fusion S	37	38
Fusion T	39	40
Fusion U	41	42
Fusion V	43	44
Xho-Eco	45	-
LEADHBACK	46	-
LEADbFOR	47	48
pRAS117	49	50
TAILbBACK	51	52
FIBRE3FOR	53	54
FIBRE6FOR	55	56
FIBRE9FOR	57	58
FIBRE12FOR	59	60
FIBRE15FOR	61	62
FIBRE18FOR	63	64
FIBRE21FOR	65	66
FIBRE22FOR	67	68
FIBREPFOR	69	70
pRAS111	71	72
MoV_H	73	74
MoV_κ	75	76
HuV_H	77	78
HuV_κ	79	80

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Imperial Cancer Research Technology Limited
(B) STREET: Sardinia House, Sardinia Street
(C) CITY: London
(E) COUNTRY: United Kingdom
(F) POSTAL CODE (ZIP): WC2A 3NL

(ii) TITLE OF INVENTION: Compounds to target cells

(iii) NUMBER OF SEQUENCES: 80

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

```
CCT CTA GTT ACC TCC AAT GTG CAG CTG CAG
 30
Pro Leu Val Thr Ser Asn Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Pro Leu Val Thr Ser Asn Val Gln Leu Gln

1 5 10

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CTC TCT CTG GAC GAG GCC GTG CAG CTG CAG
30
Leu Ser Leu Asp Glu Ala Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Leu Ser Leu Asp Glu Ala Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

```
CCT CTC AAA AAA ACC AAG GTG CAG CTG CAG
 30
Pro Leu Lys Lys Thr Lys Val Gln Leu Gln
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Pro Leu Lys Lys Thr Lys Val Gln Leu Gln
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

```
CCC CTC ACA GTT ACC TCA GTG CAG CTG CAG
 30
Pro Leu Thr Val Thr Ser Val Gln Leu Gln
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

```
CCG CTA ACC GTG CAC GAC GTG CAG CTG CAG
     30
Pro Leu Thr Val His Asp Val Gln Leu Gln
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

```
Pro Leu Thr Val His Asp Val Gln Leu Gln
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

```
CCC CTC ACA GTG TCA GAA GTG CAG CTG CAG
     30
Pro Leu Thr Val Ser Glu Val Gln Leu Gln
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

```
Pro Leu Thr Val Ser Glu Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

```
CTC ACC ACC ACC GAT AGC GTG CAG CTG CAG
 30
Leu Thr Thr Thr Asp Ser Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

```
Leu Thr Thr Thr Asp Ser Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

```
CCT CTA ACT ACT GCC ACT GTG CAG CTG CAG
     30
Pro Leu Thr Thr Ala Thr Val Gln Leu Gln
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

```
Pro Leu Thr Thr Ala Thr Val Gln Leu Gln
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

```
CCC ATT TAT ACA CAA AAT GTG CAG CTG CAG
     30
Pro Ile Tyr Thr Gln Asn Val Gln Leu Gln
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Pro Ile Tyr Thr Gln Asn Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

CAT GTA ACA GAC GAC CTA GTG CAG CTG CAG
30
His Val Thr Asp Asp Leu Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

His Val Thr Asp Asp Leu Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

```
GGT GTG ACT ATT AAT AAT GTG CAG CTG CAG
 30
Gly Val Thr Ile Asn Asn Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

```
Gly Val Thr Ile Asn Asn Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

```
GGT TTT GAT TCA CAA GGC GTG CAG CTG CAG
 30
Gly Phe Asp Ser Gln Gly Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

```
Gly Phe Asp Ser Gln Gly Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

```
AGG ATT GAT TCT CAA AAC GTG CAG CTG CAG
     30
Arg Ile Asp Ser Gln Asn Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

```
Arg Ile Asp Ser Gln Asn Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

```
TTT GAT GCT CAA AAC CAA GTG CAG CTG CAG
 30
Phe Asp Ala Gln Asn Gln Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

```
Phe Asp Ala Gln Asn Gln Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

```
CTT TTT ATA AAC TCA GCC GTG CAG CTG CAG
 30
Leu Phe Ile Asn Ser Ala Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Leu Phe Ile Asn Ser Ala Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

TCA AAC AAT TCC AAA AAC GTG CAG CTG CAG
30
Ser Asn Asn Ser Lys Asn Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

Ser Asn Asn Ser Lys Asn Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

```
GGG TTG ATG TTT GAC GCT GTG CAG CTG CAG
  30
Gly Leu Met Phe Asp Ala Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

```
Gly Leu Met Phe Asp Ala Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

```
CCT AAT GCA CCA AAC ACA GTG CAG CTG CAG
  30
Pro Asn Ala Pro Asn Thr Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Pro Asn Ala Pro Asn Thr Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

CTA GAA TTT GAT TCA AAC GTG CAG CTG CAG
30
Leu Glu Phe Asp Ser Asn Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

Leu Glu Phe Asp Ser Asn Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

```
CTT AGT TTT GAC AGC ACA GTG CAG CTG CAG
 30
Leu Ser Phe Asp Ser Thr Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

```
Leu Ser Phe Asp Ser Thr Val Gln Leu Gln
 1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

```
ATT GAT AAG CTA ACT TTG GTG CAG CTG CAG
 30
```

Ile Asp Lys Leu Thr Leu Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

Ile Asp Lys Leu Thr Leu Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Adenovirus
(B) STRAIN: Ad5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

CTCGAGTAAT AAGAATTC
18

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

AGCTAAGCTT GCATGCAAAT TC
22

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..27

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

```
CCA GCG ATG GCC AGA TCT CAG CTG CAG AGCT
 31
Pro Ala Met Ala Arg Ser Gln Leu Gln
  1               5
```

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

```
Pro Ala Met Ala Arg Ser Gln Leu Gln
  1               5
```

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 132 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 40..132

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

```
AAGCTTGCAT GCAAATTCTA TTTCAAGGAG ACAGTCATA ATG AAA TAC CTA TTG
                                    54
                                                 Met Lys Tyr Leu Leu
                                                   1               5
```

```
CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC CAA CCA GCG ATG
    102
Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala Ala Gln Pro Ala Met
                10                  15                  20

GCC AGA TCT CAG CTG CAG GTC GAC GGA TCC
    132
Ala Arg Ser Gln Leu Gln Val Asp Gly Ser
            25                  30
```

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

```
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
 1               5                  10                  15

Ala Gln Pro Ala Met Ala Arg Ser Gln Leu Gln Val Asp Gly Ser
            20                  25                  30
```

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 5..28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

```
AGCT AGA TCT ATG AAG CGC GCA AGA CCG
     28
     Arg Ser Met Lys Arg Ala Arg Pro
       1               5
```

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

Arg Ser Met Lys Arg Ala Arg Pro
1 5

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

CCT CTC AAA AAA ACC AAG CAG GTG CAG CTG CAG CAGCCTGG
41
Pro Leu Lys Lys Thr Lys Gln Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

Pro Leu Lys Lys Thr Lys Gln Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 2..34

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

C CCG CTA ACC GTG CAC GAC CAG GTG CAG CTG CAG CAGCCTGG
42
Pro Leu Thr Val His Asp Gln Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

Pro Leu Thr Val His Asp Gln Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

CCT CTA ACT ACT GCC ACT CAG GTG CAG CTG CAG CAGCCTGG
41
Pro Leu Thr Thr Ala Thr Gln Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

Pro Leu Thr Thr Ala Thr Gln Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

```
GGT GTG ACT ATT AAT AAT CAG GTG CAG CTG CAG GACCCTGG
 41
Gly Val Thr Ile Asn Asn Gln Val Gln Leu Gln
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

```
Gly Val Thr Ile Asn Asn Gln Val Gln Leu Gln
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 41 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..36

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

```
CCG TTT GAT GCT CAA AAC CAA CAG GTG CAG CTG CAG CAGCC
 41
Pro Phe Asp Ala Gln Asn Gln Gln Val Gln Leu Gln
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

Pro Phe Asp Ala Gln Asn Gln Gln Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

GGG TTG ATG TTT GAC GCT CAG GTG CAG CTG CAG CAGCC
38
Gly Leu Met Phe Asp Ala Gln Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

Gly Leu Met Phe Asp Ala Gln Val Gln Leu Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 3..35

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

```
GC CTT AGT TTT GAC AGC ACA CAG GTG CAG CTG CAG CAGCC
   40
  Leu Ser Phe Asp Ser Thr Gln Val Gln Leu Gln
   1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

```
Leu Ser Phe Asp Ser Thr Gln Val Gln Leu Gln
  1               5                  10
```

(2) INFORMATION FOR SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 50 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

```
GGA AAC AAA AAT AAT GAT AAG CTA ACT TTG CAG GTG CAG CTG CAG
   45
Gly Asn Lys Asn Asn Asp Lys Leu Thr Leu Gln Val Gln Leu Gln
  1               5                  10                  15

CAGCC
    50
```

(2) INFORMATION FOR SEQ ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 40..846

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

```
AAGCTTGCAT GCAAATTCTA TTTCAAGGAG ACAGTCATA ATG AAA TAC CTA TTG
                                                Met Lys Tyr Leu Leu
                                                 1               5

CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC CAA CCA GCG ATG
Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala Ala Gln Pro Ala Met
                  10                  15                  20

GCC CAG GTG CAG CTG CAG CAG CCT GGG GCT GAG CTT GTG AAG CCT GGG
Ala Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly
              25                  30                  35

GCT TCA GTG AAG CTG TCC TGC AAG GCT TCT GGC TAC ACC TTC ACC AGC
Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser
          40                  45                  50

TAC TGG ATG CAC TGG GTG AAG CAG AGG CCT GGA CGA GGC CTT GAG TGG
Tyr Trp Met His Trp Val Lys Gln Arg Pro Gly Arg Gly Leu Glu Trp
      55                  60                  65

ATT GGA AGG ATT GAT CCT AAT AGT GGT GGT ACT AAG TAC AAT GAG AAG
Ile Gly Arg Ile Asp Pro Asn Ser Gly Gly Thr Lys Tyr Asn Glu Lys
 70                  75                  80                  85

TTC AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA CCC TCC AGC ACA GCC
Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Pro Ser Ser Thr Ala
                  90                  95                  100

TAC ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAT
Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr
              105                 110                 115

TGT GCA AGA TAC GAT TAC TAC GGT AGT AGC TAC TTT GAC TAC TGG GGC
Cys Ala Arg Tyr Asp Tyr Tyr Gly Ser Ser Tyr Phe Asp Tyr Trp Gly
          120                 125                 130

CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGA GGC GGT TCA GGC GGA
Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
      135                 140                 145

GGT GGC TCT GGC GGT GGC GGA TCC CAG GCT GTT GTG ACT CAG GAA TCT
Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val Val Thr Gln Glu Ser
150                 155                 160                 165

GCA CTC ACC ACA TCA CCT GGT GAA ACA GTC ACA CTC ACT TGT CGC TCA
Ala Leu Thr Thr Ser Pro Gly Glu Thr Val Thr Leu Thr Cys Arg Ser
```

```
                170                 175                 180

AGT ACT GGG GCT GTT ACA ACT AGT AAC TAT GCC AAC TGG GTC CAA GAA
    630
Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala Asn Trp Val Gln Glu
            185                 190                 195

AAA CCA GAT CAT TTA TTC ACT GGT CTA ATA GGT GGT ACC AAC AAC CGA
    678
Lys Pro Asp His Leu Phe Thr Gly Leu Ile Gly Gly Thr Asn Asn Arg
        200                 205                 210

GCT CCA GGT GTT CCT GCC AGA TTC TCA GGC TCC CTG ATT GGA GAC AAG
    726
Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser Leu Ile Gly Asp Lys
    215                 220                 225

GCT GCC CTC ACC ATC ACA GGG GCA CAG ACT GAG GAT GAG GCA ATA TAT
    774
Ala Ala Leu Thr Ile Thr Gly Ala Gln Thr Glu Asp Glu Ala Ile Tyr
230                 235                 240                 245

TTC TGT GCT CTA TGG TAC AGC AAC CAC TGG GTG TTC GGT GGA GGA ACC
    822
Phe Cys Ala Leu Trp Tyr Ser Asn His Trp Val Phe Gly Gly Gly Thr
                250                 255                 260

AAA CTG ACT GTC CTA GGT CTC GAG TAATAAGAAT TC
    858
Lys Leu Thr Val Leu Gly Leu Glu
            265
```

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 269 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

```
Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Leu Ala
  1               5                  10                  15

Ala Gln Pro Ala Met Ala Gln Val Gln Leu Gln Gln Pro Gly Ala Glu
            20                  25                  30

Leu Val Lys Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly
        35                  40                  45

Tyr Thr Phe Thr Ser Tyr Trp Met His Trp Val Lys Gln Arg Pro Gly
    50                  55                  60

Arg Gly Leu Glu Trp Ile Gly Arg Ile Asp Pro Asn Ser Gly Gly Thr
65                  70                  75                  80

Lys Tyr Asn Glu Lys Phe Lys Ser Lys Ala Thr Leu Thr Val Asp Lys
                85                  90                  95

Pro Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp
            100                 105                 110

Ser Ala Val Tyr Tyr Cys Ala Arg Tyr Asp Tyr Tyr Gly Ser Ser Tyr
```

```
        115                 120                 125

Phe Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly
    130                 135                 140

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gln Ala Val
145                 150                 155                 160

Val Thr Gln Glu Ser Ala Leu Thr Thr Ser Pro Gly Glu Thr Val Thr
                165                 170                 175

Leu Thr Cys Arg Ser Ser Thr Gly Ala Val Thr Thr Ser Asn Tyr Ala
            180                 185                 190

Asn Trp Val Gln Glu Lys Pro Asp His Leu Phe Thr Gly Leu Ile Gly
        195                 200                 205

Gly Thr Asn Asn Arg Ala Pro Gly Val Pro Ala Arg Phe Ser Gly Ser
    210                 215                 220

Leu Ile Gly Asp Lys Ala Ala Leu Thr Ile Thr Gly Ala Gln Thr Glu
225                 230                 235                 240

Asp Glu Ala Ile Tyr Phe Cys Ala Leu Trp Tyr Ser Asn His Trp Val
                245                 250                 255

Phe Gly Gly Gly Thr Lys Leu Thr Val Leu Gly Leu Glu
            260                 265
```

(2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 354 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mouse

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..354

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

```
CAG GTT CAG CTG CAG CAG TCT GGA GCT GAG CTG ATG AAG CCT GGG GCC
 48
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys Pro Gly Ala
  1               5                  10                  15

TCA GTG AAG ATA TCC TGC AAG GCT ACT GGC TAC ACA TTC AGT GCC TAC
 96
Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr Phe Ser Ala Tyr
            20                  25                  30

TGG ATA GAG TGG GTA AAG CAG AGG CCT GGA CAT GGC CTT GAG TGG ATT
144
Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
```

```
          35                  40                  45

GGA GAG ATT TTA CCT GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC
    192
Gly Glu Ile Leu Pro Gly Ser Asn Asn Ser Arg Tyr Asn Glu Lys Phe
     50                  55                  60

AAG GGC AAG GCC ACA TTC ACT GCT GAT ACA TCC TCC AAC ACA GCC TAC
    240
Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
 65                  70                  75                  80

ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCC GTC TAT TAC TGT
    288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

TCA AGG TCC TAC GAC TTT GCC TGG TTT GCT TAC TGG GGC CAA GGG ACT
    336
Ser Arg Ser Tyr Asp Phe Ala Trp Phe Ala Tyr Trp Gly Gln Gly Thr
            100                 105                 110

CCG GTC ACT GTC TCT GCA
    354
Pro Val Thr Val Ser Ala
        115
```

(2) INFORMATION FOR SEQ ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

```
Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys Pro Gly Ala
 1               5                  10                  15

Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr Phe Ser Ala Tyr
            20                  25                  30

Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
        35                  40                  45

Gly Glu Ile Leu Pro Gly Ser Asn Asn Ser Arg Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
65                  70                  75                  80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
                85                  90                  95

Ser Arg Ser Tyr Asp Phe Ala Trp Phe Ala Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Pro Val Thr Val Ser Ala
        115
```

(2) INFORMATION FOR SEQ ID NO: 75:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 342 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Mouse

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..342

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

```
GAC ATT GTG ATG TCA CAG TCT CCA TCC TCC CTA GCT GTG TCA GTT GGA
 48
Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Val Gly
  1               5                  10                  15

GAG AAG GTT ACT ATG AGC TGC AAG TCC AGT CAG AGC CTT TTA TAT AGT
 96
Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
            20                  25                  30

AGC AAT CAA AAG ATC TAC TTG GCC TGG TAC CAG CAG AAA CCA GGG CAG
144
Ser Asn Gln Lys Ile Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45

TCT CCT AAA CTG CTG ATT TAC TGG GCA TCC ACT AGG GAA TCT GGG GTC
192
Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60

CCT GAT CGC TTC ACA GGC GGT GGA TCT GGG ACA GAT TTC ACT CTC ACC
240
Pro Asp Arg Phe Thr Gly Gly Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80

ATC AGC AGT GTG AAG GCT GAA GAC CTG GCA GTT TAT TAC TGT CAG CAA
288
Ile Ser Ser Val Lys Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
                85                  90                  95

TAT TAT AGA TAT CCT CGG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC
336
Tyr Tyr Arg Tyr Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
            100                 105                 110

AAA CGG
342
Lys Arg
```

(2) INFORMATION FOR SEQ ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 114 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

```
Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Val Gly
 1               5                  10                  15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
            20                  25                  30

Ser Asn Gln Lys Ile Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
        35                  40                  45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60

Pro Asp Arg Phe Thr Gly Gly Gly Ser Gly Thr Asp Phe Thr Leu Thr
65                  70                  75                  80

Ile Ser Ser Val Lys Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
                85                  90                  95

Tyr Tyr Arg Tyr Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
            100                 105                 110

Lys Arg
```

(2) INFORMATION FOR SEQ ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 354 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..354

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

```
CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC
                                                      48
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1               5                  10                  15

TCA GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC
                                                      96
Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ala Tyr
            20                  25                  30

TGG ATA GAG TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC
                                                     144
Trp Ile Glu Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
```

```
          35                  40                  45

GGA GAG ATT TTA CCT GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC
    192
Gly Glu Ile Leu Pro Gly Ser Asn Asn Ser Arg Tyr Asn Glu Lys Phe
 50                  55                  60

AAG GGC CGA GTG ACA GTC ACT AGA GAC ACA TCC ACA AAC ACA GCC TAC
    240
Lys Gly Arg Val Thr Val Thr Arg Asp Thr Ser Thr Asn Thr Ala Tyr
 65                  70                  75                  80

ATG GAG CTC AGC AGC CTG AGG TCT GAG GAC ACA GCC GTC TAT TAC TGT
    288
Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC TGG GGC CAA GGG ACT
    336
Ala Arg Ser Tyr Asp Phe Ala Trp Phe Ala Tyr Trp Gly Gln Gly Thr
           100                 105                 110

CTG GTC ACA GTC TCC TCA
    354
Leu Val Thr Val Ser Ser
       115
```

(2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

```
Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1               5                  10                  15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Ser Ala Tyr
            20                  25                  30

Trp Ile Glu Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
        35                  40                  45

Gly Glu Ile Leu Pro Gly Ser Asn Asn Ser Arg Tyr Asn Glu Lys Phe
    50                  55                  60

Lys Gly Arg Val Thr Val Thr Arg Asp Thr Ser Thr Asn Thr Ala Tyr
65                  70                  75                  80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
                85                  90                  95

Ala Arg Ser Tyr Asp Phe Ala Trp Phe Ala Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Leu Val Thr Val Ser Ser
        115
```

(2) INFORMATION FOR SEQ ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 342 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..342

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:

```
GAC ATC CAG ATG ACC CAG AGC CCA AGC AGC CTG AGC GCC AGC GTG GGT
 48
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
  1               5                  10                  15

GAC AGA GTG ACC ATC ACC TGT AAG TCC AGT CAG AGC CTT TTA TAT AGT
 96
Asp Arg Val Thr Ile Thr Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
            20                  25                  30

AGC AAT CAA AAG ATC TAC TTG GCC TGG TAC CAG CAG AAG CCA GGT AAG
144
Ser Asn Gln Lys Ile Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
        35                  40                  45

GCT CCA AAG CTG CTG ATC TAC TGG GCA TCC ACT AGG GAA TCT GGT GTG
192
Ala Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60

CCA AGC AGA TTC AGC GGT AGC GGT AGC GGT ACC GAC TTC ACC TTC ACC
240
Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr
65                  70                  75                  80

ATC AGC AGC CTC CAG CCA GAG GAC ATC GCC ACC TAC TAC TGC CAG CAA
288
Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln
                85                  90                  95

TAT TAT AGA TAT CCT CGG ACG TTC GGC CAA GGG ACC AAG GTG GAA ATC
336
Tyr Tyr Arg Tyr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
            100                 105                 110

AAA CGT
342
Lys Arg
```

(2) INFORMATION FOR SEQ ID NO: 80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 114 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

```
Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1               5                  10                  15

Asp Arg Val Thr Ile Thr Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
            20                  25                  30

Ser Asn Gln Lys Ile Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Lys
        35                  40                  45

Ala Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
    50                  55                  60

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Phe Thr
65                  70                  75                  80

Ile Ser Ser Leu Gln Pro Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln
                85                  90                  95

Tyr Tyr Arg Tyr Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile
            100                 105                 110

Lys Arg
```

CLAIMS

1. A virus, or virus-like particle, derived from a virus or virus-like particle having a receptor for a host cell comprising a modified binding specificity conferred by a binding moiety allowing the virus or virus-like particle to bind to a target cell characterised in that the said host cell receptor is modified or absent so that the virus or virus-like particle is substantially incapable of binding the said host cell.

2. A virus or virus-like particle according to Claim 1 wherein the target cell is eukaryotic.

3. A virus or virus-like particle according to Claim 2 that is an adenovirus, influenza virus, vaccinia virus, any other animal virus or replication-defective derivative of any of these.

4. An adenovirus or influenza virus or vaccinia virus, or a replication defective derivative of any of these, characterized in that the virus has a modified binding specificity conferred by a binding moiety allowing the virus to bind to a target cell.

5. A virus or virus-like particle according to any of Claims 1 to 4 wherein the binding moiety is a monoclonal antibody, an ScFv, a dAb, or a minimal recognition unit of an antibody.

6. A virus or virus-like particle according to any of Claims 1 to 4 wherein the binding moiety is at least part of a ligand of a target cell-specific cell-surface receptor.

7. A virus or virus-like particle according to Claim 5 or 6 wherein the binding moiety recognises a target cell-specific surface antigen.

8. A virus according to any one of Claims 1 to 7 wherein the binding moiety is joined to a molecule on the virus or virus-like particle other than the receptor for its host cell.

9. A virus or virus-like particle according to any one of Claims 1 to 7 wherein the binding moiety is joined to or forms part of the receptor on the said virus or virus-like particle for its host.

10. A virus or virus-like particle according to Claim 8 wherein the said molecule on the surface of the virus or virus-like particle is a protein.

11. A virus or virus-like particle according to Claim 6 wherein the target cell-specific cell-surface receptor is any one of GnRH receptor, MSH receptor and somatostatin receptor.

12. A virus or virus-like particle according to any one of Claims 1 to 11 modified further to contain a gene suitable for gene therapy.

13. A virus or virus-like particle according to Claim 12 wherein the gene encodes a molecule having a directly or indirectly cytotoxic function.

14. A virus or virus-like particle according to Claim 13 wherein the gene encodes any one of interleukin-2, tumour necrosis factor, interferon-gamma, ribonuclease and deoxyribonuclease.

15. A virus or virus-like particle according to Claim 13 wherein the gene encodes an enzyme capable of converting a relatively non-toxic pro-drug into a cytotoxic drug.

16. A virus or virus-like particle according to Claim 15 wherein the gene is either cytosine deaminase or thymidine kinase.

17. A virus or virus-like particle according to Claim 12 wherein the gene overcomes a defect in a gene in the target cell.

18. A virus or virus-like particle according to Claim 17 wherein the gene is any one of CFTR, dystrophin and haemoglobin A.

19. A virus, or virus-like particle, containing nucleic acid, according to any one of Claims 1 to 15 wherein the said virus or virus-like particle is adapted to deliver the said nucleic acid to the target cell.

20. A virus or virus-like particle according to Claim 1 wherein the said receptor comprises protein.

21. A virus according to Claim 20 wherein the virus is influenza virus and the said receptor is the haemagglutinin receptor protein.

22. A virus according to Claim 20 wherein the virus is adenovirus and the said receptor is the penton fibre protein.

23. A virus according to Claim 22 wherein the binding moiety is fused to the penton fibre protein at any one or more of the junctions of the repetitive units of the shaft.

24. A virus according to Claim 23 wherein the binding moiety is a ScFv.

25. A virus according to Claim 24 wherein the ScFv binds to a tumour cell antigen.

26. A virus or virus-like particle according to any one of Claims 1 to 25 wherein the binding moiety is a polypeptide.

27. A virus or virus-like particle according to Claim 26 when dependent on either of Claims 10 or 20 wherein the binding moiety is fused to the protein on the surface of the said virus or virus-like particle.

28. A virus or virus-like particle according to any one of Claims 1 to 27 for use in medicine.

29. A nucleotide sequence encoding the fusion of the binding moiety and the protein on the surface of the virus or virus-like particle according to any one of Claims 23 to 25 and 27.

30. A nucleotide sequence encoding the receptor modified as defined in Claim 8, wherein the receptor comprises a polypeptide backbone.

31. A nucleotide sequence defined in any of Claims 29 or 30 additionally comprising the remainder of the genome of the virus or virus-like particle.

32. A nucleotide sequence encoding a virus or virus-like particle

according to any one of Claims 1 to 27.

33. A therapeutic system comprising a virus or virus-like particle according to Claim 15 or 16 and a pro-drug.

34. A method for producing a virus or virus-like particle according to any of Claims 1 to 27 in cell culture, the method comprising (1) infecting the cells with the said virus or virus-like particle, (2) culturing the infected cells until the virus or virus-like particle reaches a sufficiently high titre, (3) harvesting and substantially purifying the virus or virus-like particle and (4) joining the binding moiety to the substantially purified virus or virus-like particle.

35. A method for producing a virus or virus-like particle according to any of Claims 1 to 27 in cell culture, the method comprising (1) genetically modifying the virus or virus-like particle to produce a binding moiety, (2) infecting cells with the genetically modified virus or virus-like particle, (3) culturing the cells until the virus or virus-like particle reaches a sufficiently high titre and (4) harvesting and substantially purifying the genetically modified virus or virus-like particle.

36. A pharmaceutical composition comprising a virus or virus-like particle according to any one of Claims 1 to 27 and a pharmaceutical carrier.

37. A method of treating a mammal having target cells to be destroyed, the method comprising administering the virus or virus-like particle according to Claim 13.

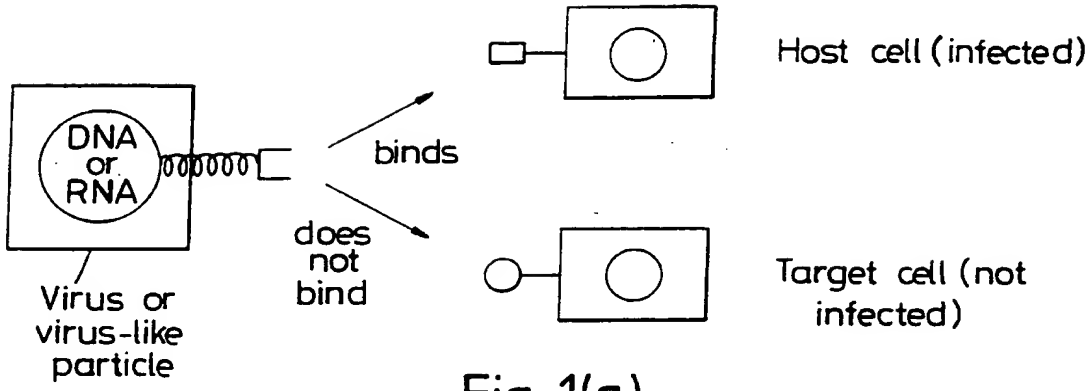


Fig. 1(a)

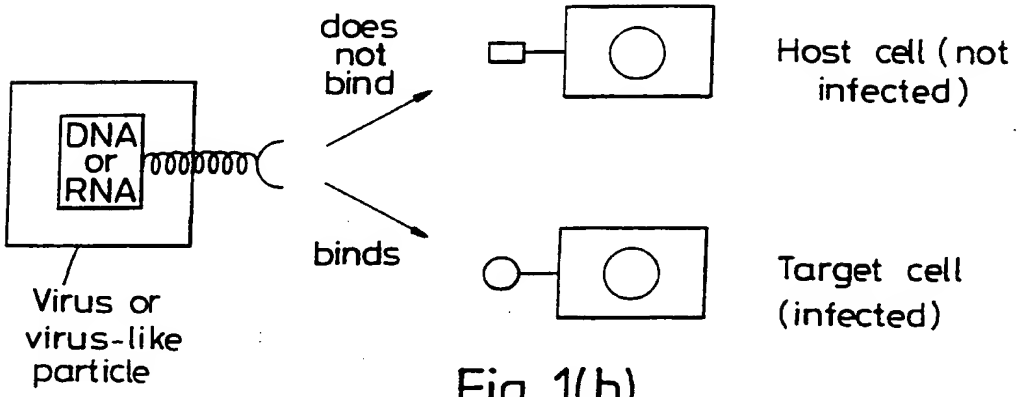


Fig. 1(b)

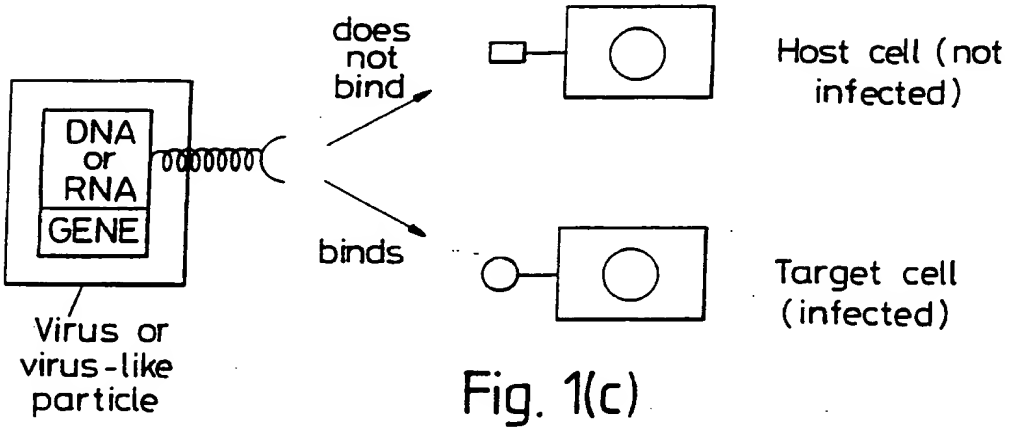


Fig. 1(c)

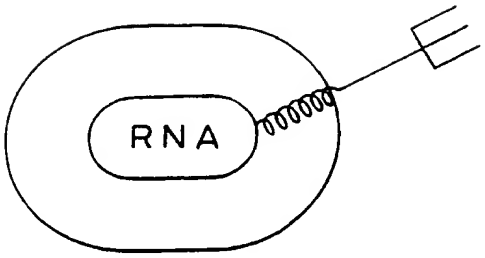


Fig. 3(a)

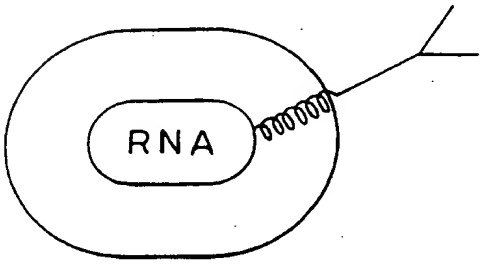


Fig. 3(b)

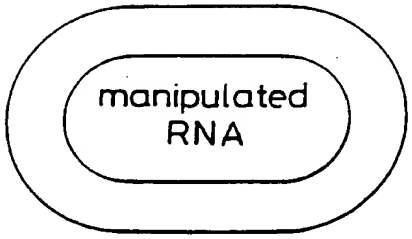


Fig. 4(a)

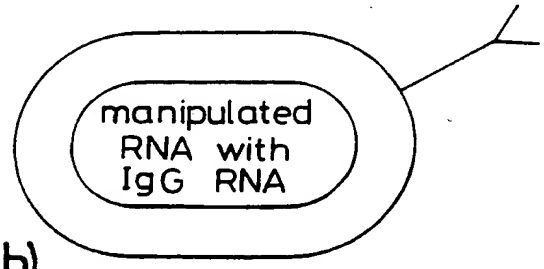


Fig. 4(b)

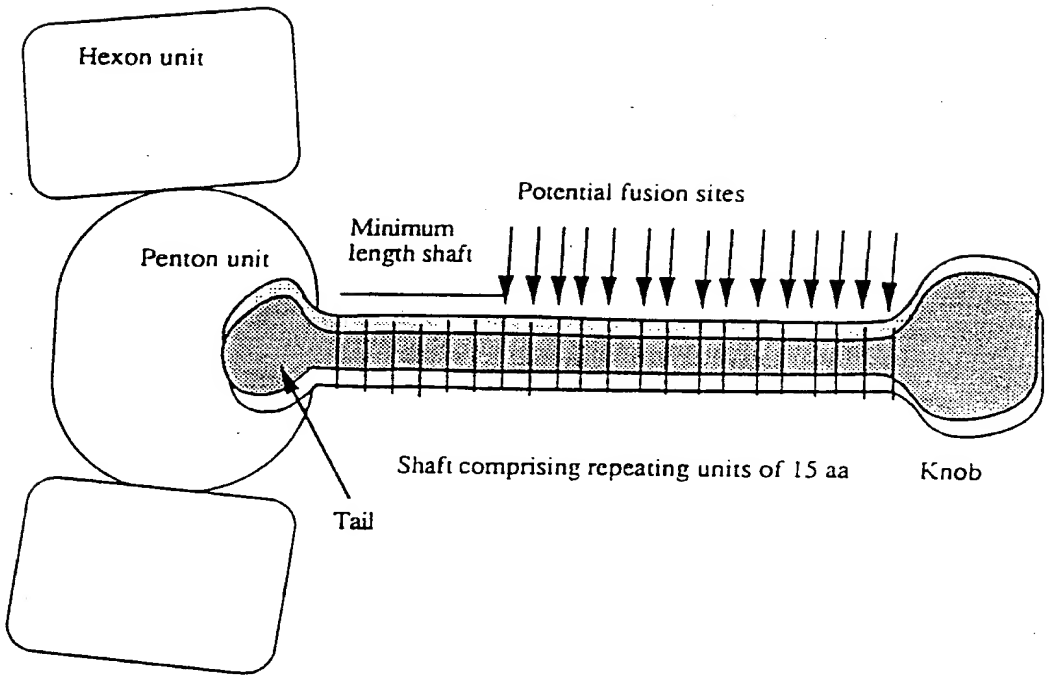

Hexon unit
Penton unit
Potential fusion sites
Minimum length shaft
Shaft comprising repeating units of 15 aa
Knob
Tail

Figure 5

Fusion A

```
                    1  2  3  4            108 109
 P  L  V  T  S  N  V  Q  L  Q ..........L  E  *  *
CCTCTAGTTACCTCCAATGTGCAGCTGCAG...ScFv...CTCGAGTAATAAGAATTC
     31210         |       PstI ..         .XhoI        EcoRI
```

Fusion B

```
                    1  2  3  4            108 109
 L  S  L  D  E  A  V  Q  L  Q ..........L  E  *  *
CTCTCTCTGGACGAGGCCGTGCAGCTGCAG...ScFv...CTCGAGTAATAAGAATTC
       31260       |       PstI ..         .XhoI        EcoRI
```

Fusion C

```
                    1  2  3  4            108 109
 P  L  K  K  T  K  V  Q  L  Q ..........L  E  *  *
CCTCTCAAAAAAACCAAGGTGCAGCTGCAG...ScFv...CTCGAGTAATAAGAATTC
31310     31320     |       PstI ..         .XhoI        EcoRI
```

Fusion D

```
                    1  2  3  4            108 109
 P  L  T  V  T  S  V  Q  L  Q ..........L  E  *  *
CCCCTCACAGTTACCTCAGTGCAGCTGCAG...ScFv...CTCGAGTAATAAGAATTC
     31360         |       PstI ..         .XhoI        EcoRI
```

Fusion E

```
                    1  2  3  4            108 109
 P  L  M  V  A  D  V  Q  L  Q ..........L  E  *  *
CCTCTAATGGTCGCGGGCGTGCAGCTGCAG...ScFv...CTCGAGTAATAAGAATTC
31400     31410     |       PstI ..         .XhoI        EcoRI
```

Figure 6 (Page 1 of 5)

Fusion P

```
                        1  2  3  4          108 109
 P  F  I  N  S  A  V  Q  L  Q ..........L  E  *  *
CTTTTTATAAACTCAGCCGTGCAGCTGCAG...ScFv...CTCGAGTAATAAGAATTC
    31920          |       PstI ..        .XhoI        EcoRI
```

Fusion Q

```
                        1  2  3  4          108 109
 S  N  N  S  K  N  V  Q  L  Q ..........L  E  *  *
TCAAACAATTCCAAAAACGTGCAGCTGCAG...ScFv...CTCGAGTAATAAGAATTC
   32030      32040|       PstI ..        .XhoI        EcoRI
```

Fusion R

```
                        1  2  3  4          108 109
 G  L  M  F  D  A  V  Q  L  Q ..........L  E  *  *
GGGTTGATGTTTGACGCTGTGCAGCTGCAG...ScFv...CTCGAGTAATAAGAATTC
   32030      32040|       PstI ..        .XhoI        EcoRI
```

Fusion S

```
                        1  2  3  4          108 109
 P  N  A  P  N  T  V  Q  L  Q ..........L  E  *  *
CCTAATGCACCAAACACAGTGCAGCTGCAG...ScFv...CTCGAGTAATAAGAATTC
          32100    |       PstI ..        .XhoI        EcoRI
```

Fusion T

```
                        1  2  3  4          108 109
 L  E  F  D  S  N  V  Q  L  Q ..........L  E  *  *
CTAGAATTTGATTCAAACGTGCAGCTGCAG...ScFv...CTCGAGTAATAAGAATTC
          32150 |       PstI ..        .XhoI        EcoRI
```

Figure 6 (Page 4 of 5)

LEADHBACK AGCTAAGCTTGCATGCAAATTC

*Hin*dIII *Sph*I

LEADbFOR *pel*B leader|

P A M A R S Q L Q

CCAGCGATGGCCAGATCTCAGCTGCAGAGCT

*Bgl*II *Pst*I

Figure 7

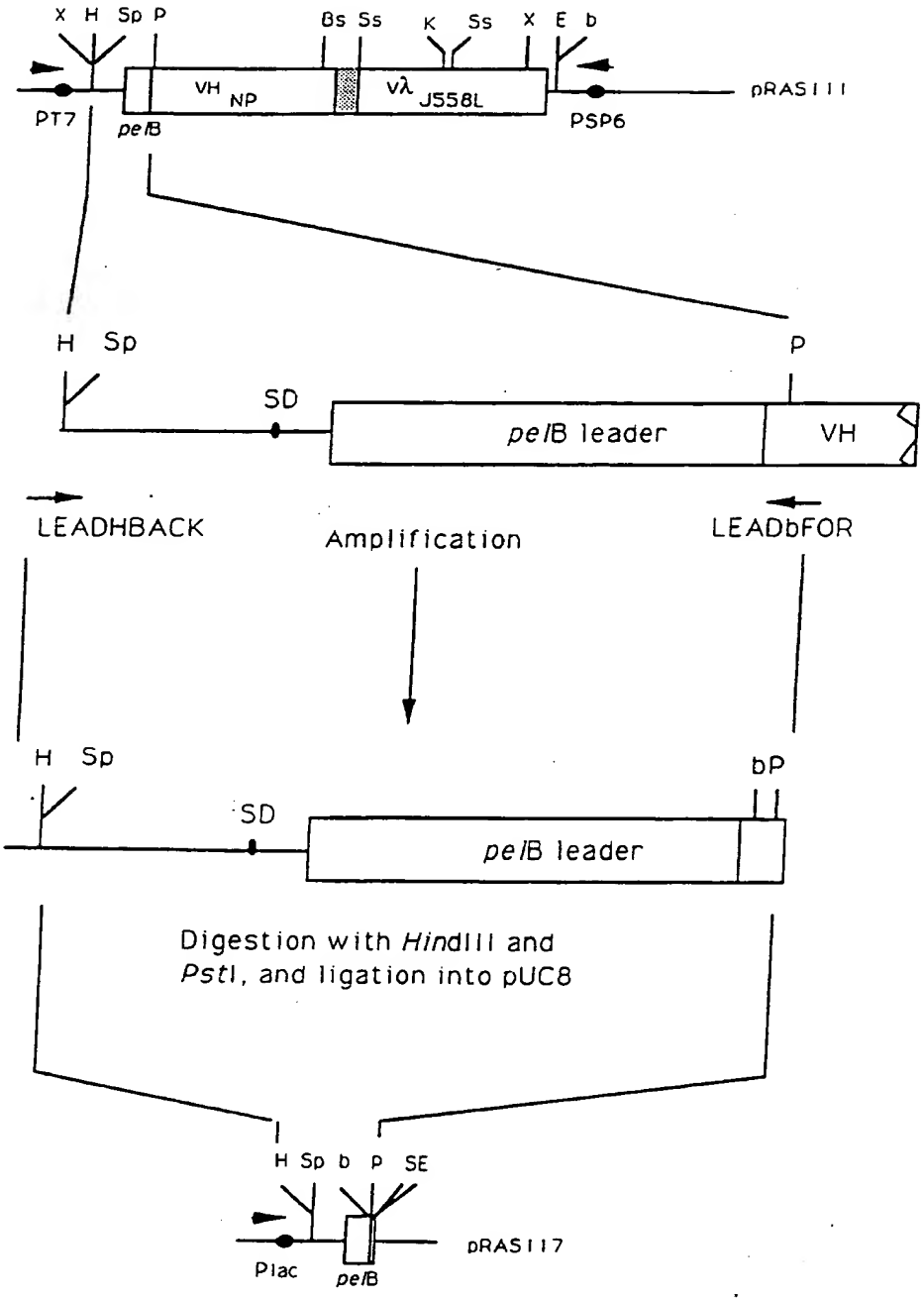

X H Sp P
Bs Ss
K Ss
X E b
VH NP
Vλ J558L
pRAS111
PT7
pelB
PSP6
H Sp
P
SD
pelB leader
VH
LEADHBACK
Amplification
LEADbFOR
H Sp
bP
SD
pelB leader
Digestion with HindIII and
PstI, and ligation into pUC8
H Sp b P SE
pRAS117
Plac
pelB

Figure 8

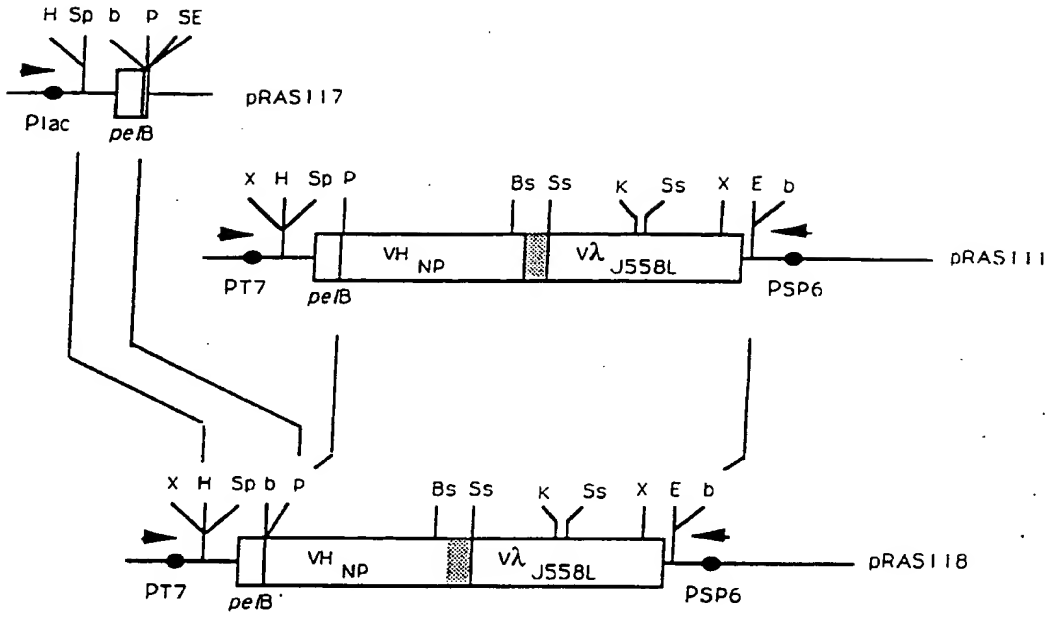

H Sp b P SE
Plac
pelB
pRAS117
X H Sp P
Bs Ss
K Ss
X E b
VH NP
Vλ J558L
PT7
pelB
PSP6
pRAS111
X H Sp b P
Bs Ss
K Ss
X E b
VH NP
Vλ J558L
PT7
pelB
PSP6
pRAS118

Figure 11

FIBRE18FOR --fibre----------/--scFv-------

G L M F D G Q V Q L Q

GGGTTGATGTTTGACGCTCAGGTGCAGCTGCAGCAGCC

PstI

FIBRE21FOR --fibre----------/--scFv-------

L S F D S T Q V Q L Q

GCCTTAGTTTTGACAGCACACAGGTGCAGCTGCAGCAGCC

PstI

FIBRE22FOR --fibre----------/--scFv-------

G N K N N D K L T L Q V Q L Q

GGAAACAAAAATAATGATAAGCTAACTTTGCAGGTGCAGCTGCAGCAGCC

PstI

FIBREPFOR --fibre----------

Y I A Q E *

CATACATTGCCCAAGAATAACAGGTGCAGCTGCAGCAGCCTGG

PstI

Figure 12 (Page 2 of 2)

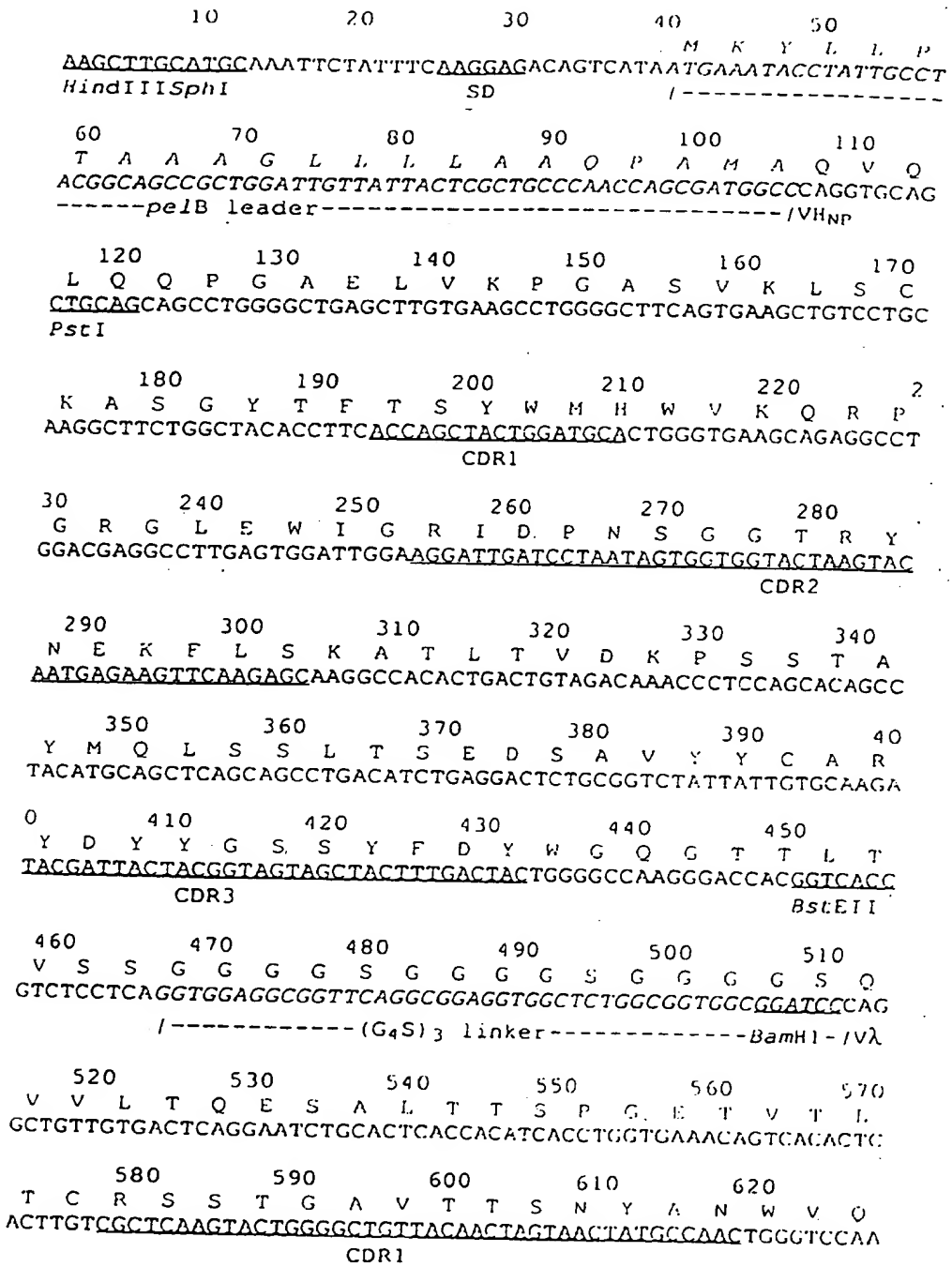
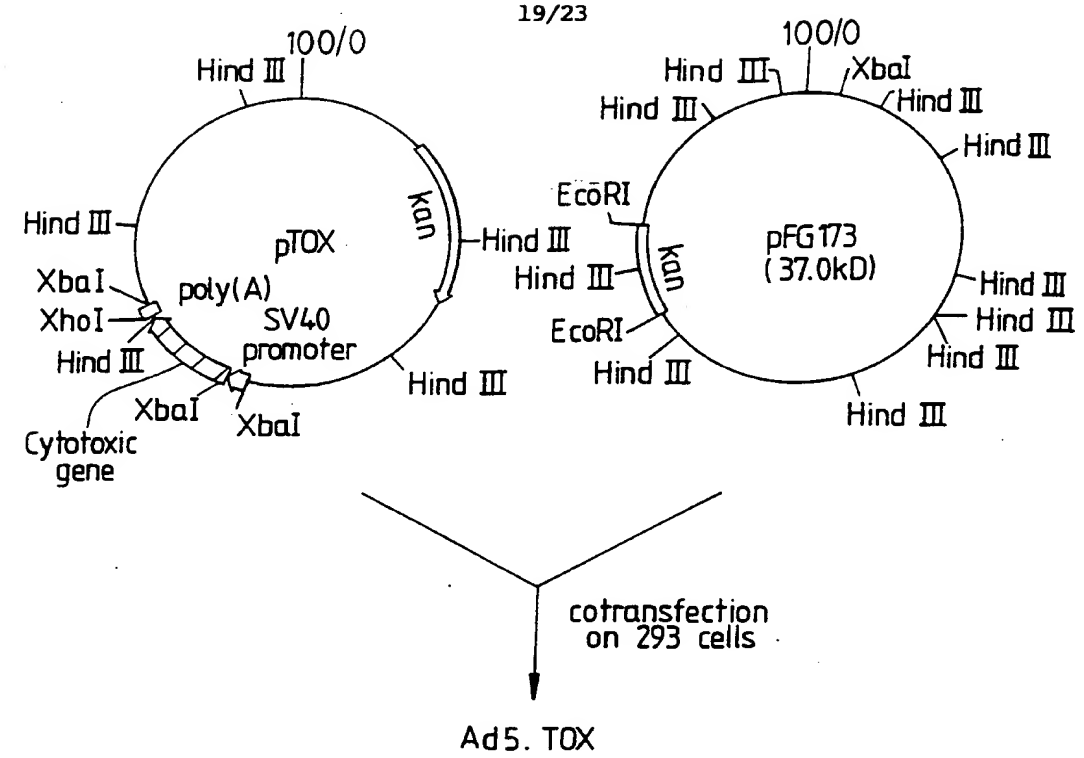


Figure 13 (Page 1 of 2)


100/0
Hind III
pTOX
kan
poly(A)
SV40
promoter
XbaI
XhoI
Cytotoxic
gene
EcoRI
pFG173
(37.0kD)
cotransfection
on 293 cells
Ad5. TOX

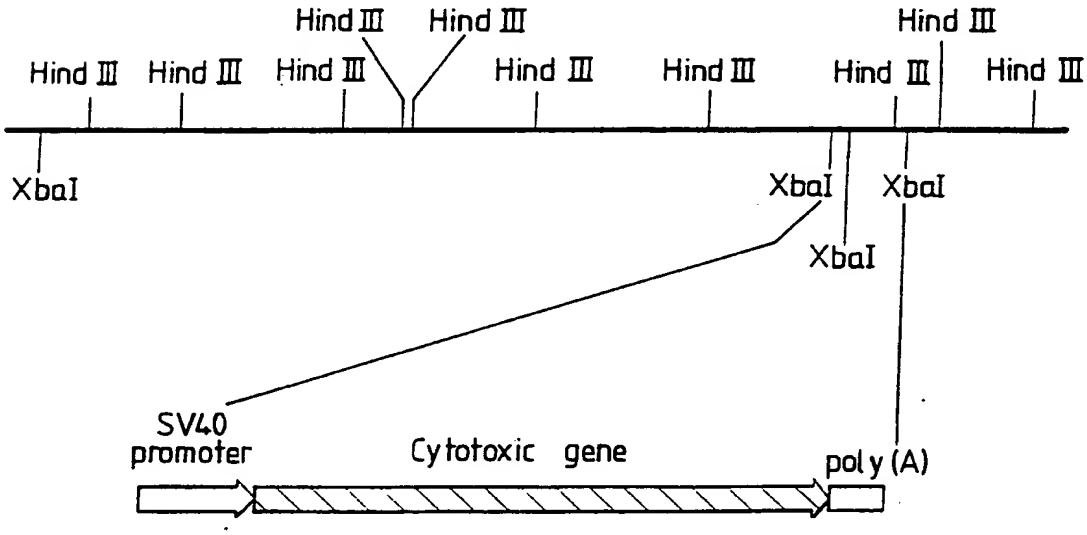

Hind III
XbaI
SV40
promoter
Cytotoxic gene
poly (A)

Fig. 14

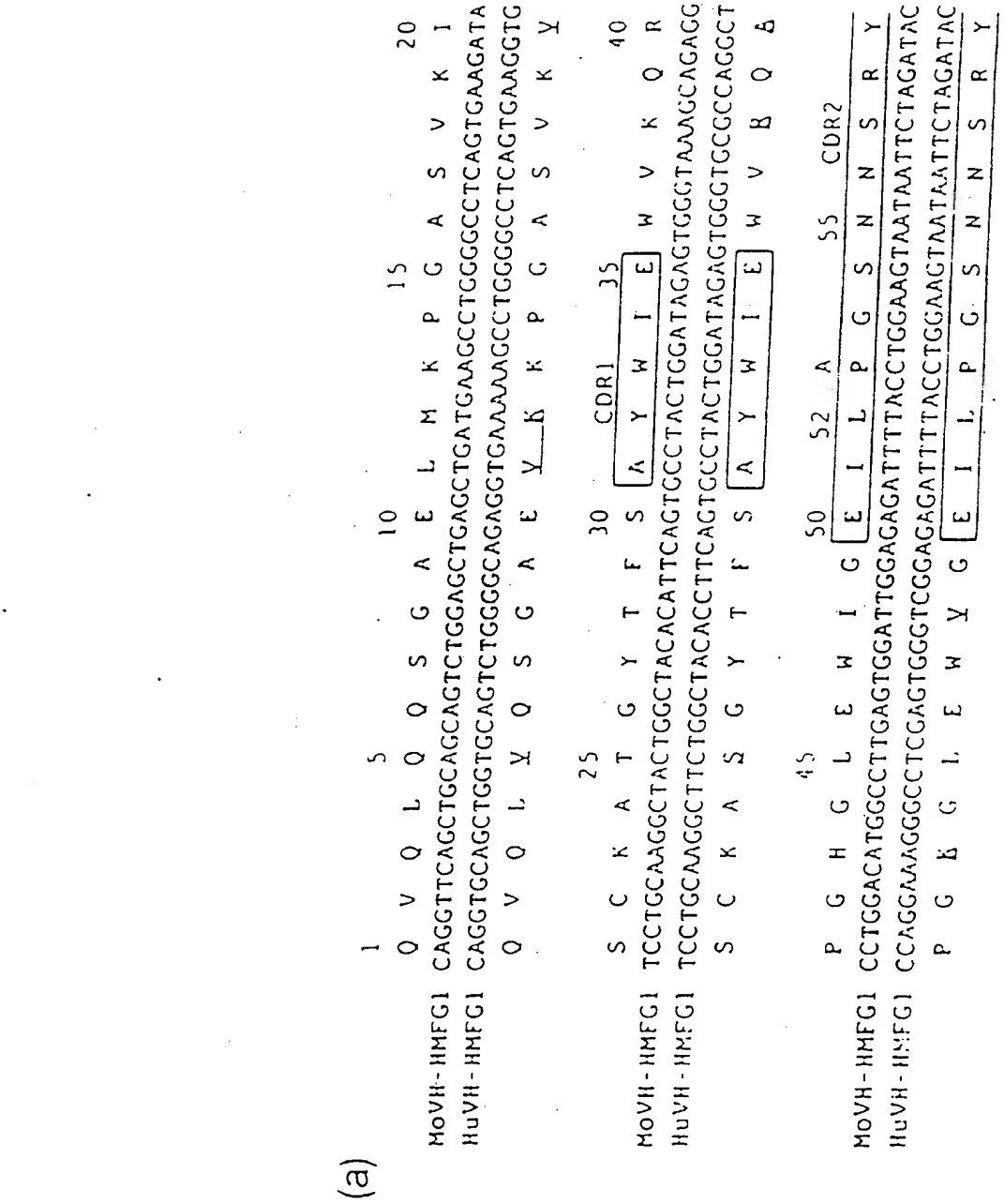


Figure 15 (Page 1 of 4)

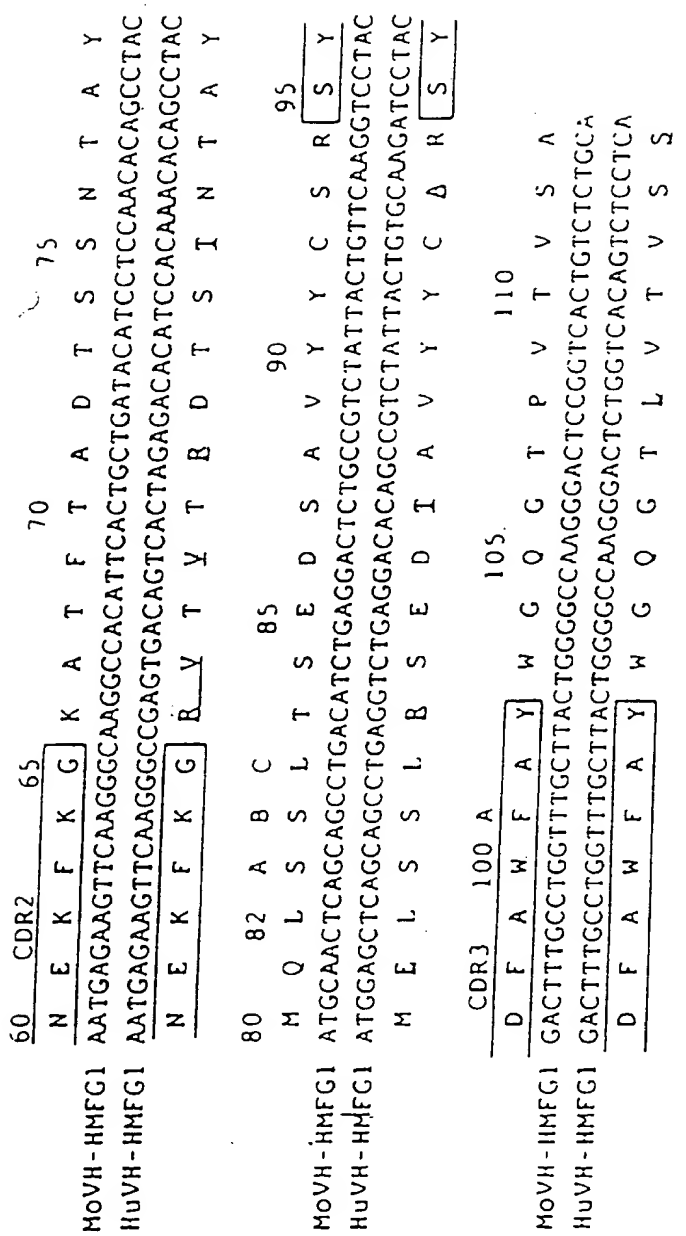


Figure 15 (Page 2 of 4)

(b)

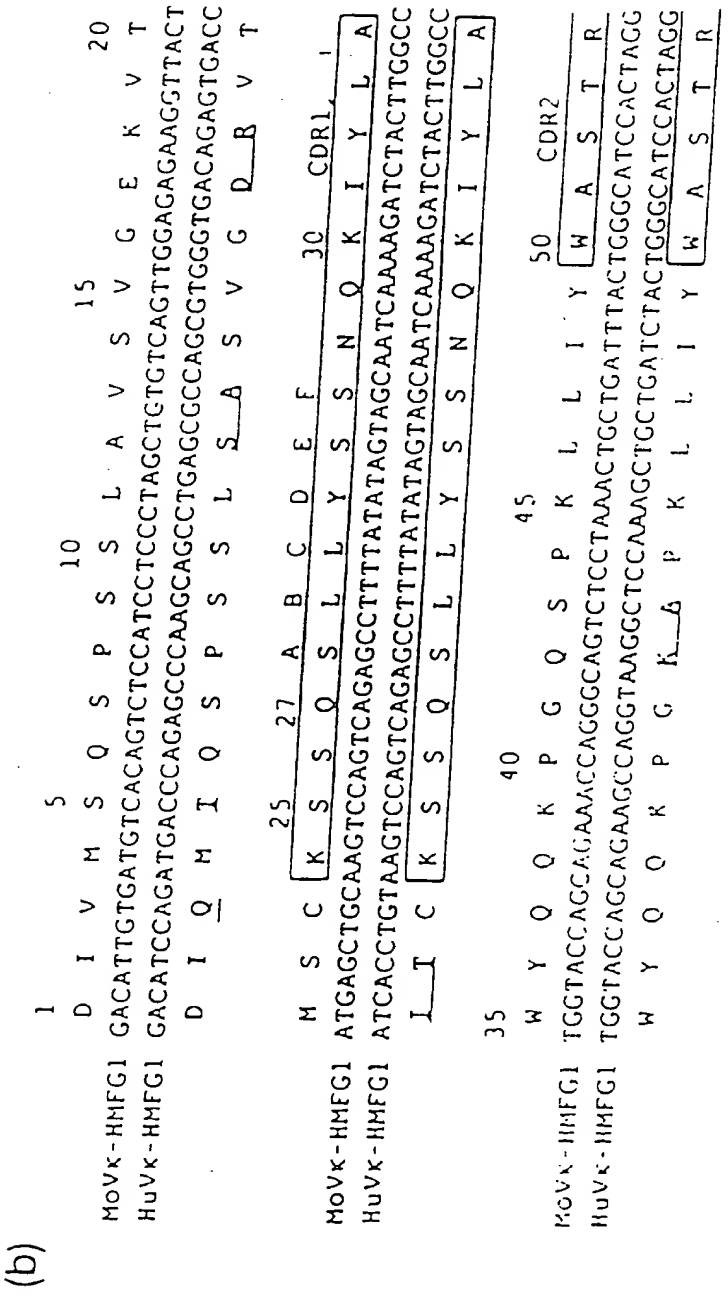


Figure 15 (Page 3 of 4)

INTERNATIONAL SEARCH REPORT

Internati l Application No
PCT/GB 93/02267

A. CLASSIFICATION OF SUBJECT MATTER
IPC 5 C12N15/87 A61K48/00 C12N15/34 C12N15/86 C12N7/04
C12N15/62 C12N15/13

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 5 C12N A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	NUCLEIC ACIDS RESEARCH. vol. 21, no. 5 , 11 March 1993 , ARLINGTON, VIRGINIA US pages 1081 - 1085 RUSSELL, S.J. ET AL. 'Retroviral vectors displaying functional antibody fragments' cited in the application see the whole document ---	1-7,9, 10,12, 13,15, 19,20, 26-29, 31-38
X	HUMAN GENE THERAPY vol. 3, no. 2 , April 1992 pages 147 - 154 CURIEL, D.T. ET AL. 'High-efficiency gene transfer mediated by adenovirus coupled to DNA-polylysine complexes' cited in the application see the whole document --- -/--	1-3,6,8, 10,12, 17,19, 20,26, 28,33, 34,36,37

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

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Date of the actual completion of the international search	Date of mailing of the international search report
7 February 1994	04 -03- 1994
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Chambonnet,. F

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INTERNATIONAL SEARCH REPORT

Internat' Application No
PCT/GB 93/02267

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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